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(57) Abstract

Internal Transcribed Spacer (ITS) DNA sequences from the ribosomal RNA gene region are described for different species and strains of wheat fungal pathogens, including *Fusarium* spp. and *Microdochium nivale*. Specific primers from within these sequences are identified as being useful for the identification of the fungal isolates using PCR–based techniques.

DETECTION OF WHEAT FUNGAL PATHOGENS USING THE POLYMERASE CHAIN REACTION

The present invention relates to the use of species-specific primers in polymerase chain reaction assays for the detection of fungal pathogens of wheat. The use of these primers enables the detection of specific isolates of fungal pathogens and the monitoring of disease development in plant populations.

There is a real need for the development of technology that will allow the identification of specific races of pathogen fungi early in the infection process. By identifying the specific race of a pathogen before disease symptoms become evident in the crop stand, the agriculturist can assess the likely effects of further development of the pathogen in the crop variety in which it has been identified and can choose an appropriate fungicide if such application is deemed necessary.

The present invention is drawn to methods of identification of different pathotypes of plant pathogenic fungi. The invention provides Internal Transcribed Spacer (ITS) DNA sequences that show variability between different fungal pathotypes. Such DNA sequences are useful in the method of the invention as they can be used to derive primers for use in polymerase chain reaction (PCR)-based diagnostic assays. These primers generate unique fragments in PCR reactions in which the DNA template is provided by specific fungal pathotypes and can thus be used to identify the presence or absence of specific pathotypes in host plant material before the onset of disease symptoms.

In a preferred embodiment, the invention provides ITS1 and ITS2 DNA sequences for the pathogens *Fusarium poae*, *Fusarium avenaceum*, and *Microdochium nivale*. In another preferred embodiment, the invention provides ITS-derived diagnostic primers for the detection of *Fusarium* spp. and *Microdochium nivale*.

This invention provides the possibility of assessing potential damage in a specific crop variety-pathogen strain relationship and of utilizing judiciously the diverse armory of fungicides that is available. Furthermore, the invention can be used to provide detailed information on the development and spread of specific pathogen races over extended geographical areas. The invention provides a method of detection that is especially suitable for diseases with a long latent phase.

Kits useful in the practice of the invention are also provided. The kits find particular use in the identification of the fungal pathogens *Fusarium* spp. and *Microdochium nivale.*

The present invention provides unique DNA sequences that are useful in identifying different pathotypes of plant pathogenic fungi. Particularly, the DNA sequences can be used as primers in PCR-based analysis for the identification of fungal pathotypes. The DNA sequences of the invention include the Internal Transcribed Spacer (ITS) sequences of the ribosomal RNA gene regions of particular fungal pathogens as well as primers derived from these regions that are capable of identifying the particular pathogen. These ITS DNA sequences from different pathotypes within a pathogen species or genus, which vary between the different members of the species or genus, can be used to identify those specific members.

Biomedical researchers have used PCR-based techniques for some time and with moderate success to detect pathogens in infected animal tissues. Only recently, however, has this technique been applied to detect plant pathogens. The presence of *Gaumannomyces graminis* in infected wheat has been detected using PCR of sequences specific to the pathogen mitochondrial genome (Schlesser *et al.*, 1991; *Applied and Environ. Microbiol.* 57: 553-556), and random amplified polymorphic DNA (*i.e.* RAPD) markers were able to distinguish numerous races of *Gremmeniella abietina*, the causal agent of scleroderris canker in conifers. U.S. Patent No. 5,585,238 (herein incorporated by reference in its entirety) describes primers derived from the ITS sequences of the ribosomal RNA gene region of strains of *Septoria, Pseudocercosporella,* and *Mycosphaerella* and their use in the identification of these fungal isolates using PCR-based techniques. In addition, WO 95/29260 (herein incorporated by reference in its entirety) describes primers derived from the ITS sequences of the ribosomal RNA gene region of strains of *Fusarium* and their use in the identification of these fungal isolates using PCR-based techniques. Furthermore, European Patent Application No. 97810779.5 (herein incorporated by reference in its entirety) describes primers derived from the ITS sequences of the ribosomal RNA gene region of strains of *Cercospora, Helminthosporium, Kabatiella, and Puccinia* and their use in the identification of these fungal isolates using PCR-based techniques.

Ribosomal genes are suitable for use as molecular probe targets because of their high copy number. Despite the high conservation between mature rRNA sequences, the non-transcribed and transcribed spacer sequences are usually poorly conserved and are

thus suitable as target sequences for the detection of recent evolutionary divergence. Fungal rRNA genes are organized in units, each of which encodes three mature subunits of 18S (small subunit), 5.8S, and 28S (large subunit). These subunits are separated by two Internal Transcribed Spacers, ITS1 and ITS2, of around 300 bp (White *et al.*, 1990; In: PCR Protocols; Eds.: Innes *et al.*; pages 315-322). In addition, the transcriptional units are separated by non-transcribed spacer sequences (NTSs). The ITS and NTS sequences are particularly suitable for the detection of specific pathotypes of different fungal pathogens.

The DNA sequences of the invention are from the Internal Transcribed Spacer sequences of the ribosomal RNA gene region of different plant pathogens. The ITS DNA sequences from different pathotypes within a pathogen species or genus vary among the different members of the species or genus. Once having determined the ITS sequences of a pathogen, these sequences can be aligned with other ITS sequences. In this manner, primers can be derived from the ITS sequences. That is, primers can be designed based on regions within the ITS sequences that contain the greatest differences in sequence among the fungal pathotypes. These sequences and primers based on these sequences can be used to identify specific pathogens.

Particular DNA sequences of interest include ITS DNA sequences from *Fusarium poae, Microdochium nivale,* and *Fusarium avenaceum.* Such ITS DNA sequences are disclosed in SEQ ID NOs: 22-24, respectively. Sequences of representative oligonucleotide primers derived from these ITS sequences are disclosed in SEQ ID NOs: 7-18. The sequences find use in the PCR-based identification of the pathotypes of interest.

Encompassed by the present invention is an isolated Internal Transcribed Spacer sequence selected from the group consisting of ITS1 of *Fusarium poae* comprising nucleotides 31-180 of SEQ ID NO:22, ITS2 of *Fusarium poae* comprising nucleotides 338-489 of SEQ ID NO: 22, ITS1 of *Microdochium nivale* comprising nucleotides 31-175 of SEQ ID NO:23, ITS2 of *Microdochium nivale* comprising nucleotides 333-499 of SEQ ID NO:23, ITS1 of *Fusarium avenaceum* comprising nucleotides 31-181 of SEQ ID NO:24, and ITS2 of *Fusarium avenaceum* comprising nucleotides 339-504 of SEQ ID NO:24.

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Methods for the use of the primer sequences of the invention in PCR analysis are well known in the art. For example, see U.S. Patent Nos. 4,683,195 and 4,683,202, as well as Schlesser *et al.* (1991) *Applied and Environ. Microbiol. 57*:553-556. See also, Nazar *et al.* (1991; *Physiol. and Molec. Plant Pathol.* 39: 1-11), which used PCR amplification to exploit differences in the ITS regions of *Verticillium albo-atrum* and *Verticillium dahliae* and therefore distinguish between the two species; and Johanson and Jeger (1993; *Mycol. Res.* 97: 670-674), who used similar techniques to distinguish the banana pathogens *Mycosphaerella fijiensis* and *Mycospharella musicola.*

The ITS DNA sequences of the invention can be cloned from fungal pathogens by methods known in the art. In general, the methods for the isolation of DNA from fungal isolates are known. See, Raeder & Broda (1985) *Letters in Applied Microbiology 2*:17-20; Lee *et al.* (1990) *Fungal Genetics Newsletter 35*:23-24; and Lee and Taylor (1990) In: *PCR Protocols: A Guide to Methods and Applications*, Innes *et al.* (Eds.); pages 282-287.

Alternatively, the ITS sequences of interest can be determined by PCR amplification. In an exemplified embodiment, primers to amplify the entire ITS region were designed according to White *et al.* (1990; In: PCR Protocols; Eds.: Innes *et al.* pages 315-322), and the amplified ITS sequence was subcloned into the pCRII cloning vector. The subcloned sequence included the left hand ITS (ITS1), the right hand ITS (ITS2), as well as the centrally located 5.8S rRNA gene. This was undertaken for several species of *Fusarium*, including *Fusarium poae* and *Fusarium avenaceum*, as well as *Microdochium nivale.*

The determined ITS sequences were compared within each pathogen group to locate divergences that might be useful to test in PCR to distinguish the different species and/or strains. The ITS DNA sequences that were determined are shown in SEQ ID NOs: 19-24 and the comparative alignment is shown in FIGURE 1. From the identification of divergences, numerous primers were synthesized and tested in PCR-amplification. Templates used for PCR-amplification testing were firstly purified pathogen DNA, and subsequently DNA isolated from infected host plant tissue. Thus, it was possible to identify pairs of primers that were diagnostic, *i.e.* that identified one particular pathogen species or strain but not another species or strain of the same pathogen. Primers were also designed

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to regions highly conserved among the species to develop genus-specific primers as well as primers that will identify any of several fungal pathogens that cause a particular disease. For example, primers were developed to detect Fusarium caused by various fungal pathogens, including *Fusarium spp.* and *Microdochium nivale.*

Preferred primer combinations are able to distinguish between the different species or strains in infected host tissue, *i.e.* host tissue that has previously been infected with a specific pathogen species or strain. This invention provides numerous primer combinations that fulfill this criterion for different *Fusarium* spp. and *Microdochium nivale.* The primers of the invention are designed based on sequence differences among the fungal ITS regions. A minimum of one base pair difference between sequences can permit design of a discriminatory primer. Primers designed to a specific fungal DNA's ITS region can be used in combination with a primer made to a conserved sequence region within the ribosomal DNA's coding region to amplify species-specific PCR fragments. In general, primers should have a theoretical melting temperature between about 60 to about 70 degree °C to achieve good sensitivity and should be void of significant secondary structure and 3' overlaps between primer combinations. Primers generally have sequence identity with at least about 5-10 contiguous nucleotide bases of ITS1 or ITS2, preferably with at least 10 contiguous nucleotide bases of ITS1 or ITS2. In preferred embodiments, primers are anywhere from approximately 5-30 nucleotide bases long.

Preferred is within the scope of the invention is an oligonucleotide primer for use in identification of a fungal pathogen, wherein said oligonucleotide primer is selected from the group consisting of SEQ ID NO's:7-15.

A further embodiment of the present invention is a pair of oligonucleotide primers for use in the amplification-based detection of a fungal Internal Transcribed Spacer DNA sequence, wherein at least one of said primers is the oligonucleotide primer according to the invention. Preferred is a pair of oligonucleotide primers, wherein said pair is selected from the following primer pairs:

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SEQ ID NO:13 and SEQ ID NO:4;
SEQ ID NO:14 and SEQ ID NO:11;
SEQ ID NO:15 and SEQ ID NO:11;
SEQ ID NO:14 and SEQ ID NO:4;
SEQ ID NO:15 and SEQ ID NO:4;
SEQ ID NO12 and SEQ ID NO:10;
SEQ ID NO:12 and SEQ ID NO:4;
SEQ ID NO:1 and SEQ ID NO:10;
SEQ ID NO:1 and SEQ ID NO:11;
SEQ ID NO:7 and SEQ ID NO:11;
SEQ ID NO:8 and SEQ ID NO:11;
SEQ ID NO:9 and SEQ ID NO:11;
SEQ ID NO:7 and SEQ ID NO:4;
SEQ ID NO:7 and SEQ ID NO:16;
SEQ ID NO:7 and SEQ ID NO:18; and
SEQ ID NO:7 and SEQ ID NO:17.

A preferred pair of oligonucleotide primers used for detecting *Microdochium nivale* is selected from the following primer pairs:

SEQ ID NO:13 and SEQ ID NO:4;
SEQ ID NO:14 and SEQ ID NO:11;
SEQ ID NO:15 and SEQ ID NO:11;
SEQ ID NO:14 and SEQ ID NO:4;
SEQ ID NO:15 and SEQ ID NO:4;
SEQ ID NO:12 and SEQ ID NO:10;
SEQ ID NO:12 and SEQ ID NO:4;
SEQ ID NO:1 and SEQ ID NO:10;
SEQ ID NO:1 and SEQ ID NO:11;
SEQ ID NO:7 and SEQ ID NO:11;
SEQ ID NO:8 and SEQ ID NO:11; and
SEQ ID NO:9 and SEQ ID NO:11.

A preferred pair of oligonucleotide primers used for detecting *Microdochium nivale*, *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, and *F. moniliforme* is SEQ ID

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NO:7 and SEQ ID NO:4.

A preferred pair of oligonucleotide primers used for detecting *F. graminearum, F. moniliforme, F. roseum, F. poae*, and *F culmorum* is SEQ ID NO:7 and SEQ ID NO:16.

A preferred pair of oligonucleotide primers used for detecting *F. graminearum* and *F. culmorum* is selected from:

SEQ ID NO:7 and SEQ ID NO:18; and

SEQ ID NO:7 and SEQ ID NO:17.

The present invention relates to method for the detection of a fungal pathogen selected from the group consisting of *Fusarium poae, Microdochium nivale* and *Fusarium avenaceum*, comprising the steps of:

(a) isolating DNA from a plant leaf infected with said pathogen;

(b) subjecting said DNA to polymerase chain reaction amplification using at least one primer having at least 10 contiguous nucleotides which are identical to at least 10 contiguous nucleotides of the Internal Transcribed Spacer sequences of nucleotides 31-180 of SEQ ID NO:22, nucleotides 338-489 of SEQ ID NO:22, nucleotides 31-175 of SEQ ID NO:23, nucleotides 333-499 of SEQ ID NO:23, or nucleotides 339-504 of SEQ ID NO:24; and

(c) detecting said fungal pathogen by visualizing the product or products of said polymerase chain reaction amplification.

Preferred is said method for the detection of a fungal pathogen, wherein said fungal pathogen is selected from *Fusarium poae* and *Microdochium nivale*.

Further encompassed by the present invention is a method for the detection of *Fusarium avenaceum*, comprising the steps of:

(a) isolating DNA from a plant leaf infected with *Fusarium avenaceum*:

(b) subjecting said DNA to polymerase chain reaction amplification using at least one primer having at least 10 contiguous nucleotides identical to at least 10 contiguous nucleotides of nucleotides 31-118 or 138-181 of SEQ ID NO:24; and

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(c) detecting *Fusarium avenaceum* by visualizing the product or products of said polymerase chain reaction amplification.

The present invention lends itself readily to the preparation of "kits" containing the elements necessary to carry out the process. Such a kit may comprise a carrier being compartmentalized to receive in close confinement therein one or more container, such as tubes or vials. One of the containers may contain unlabeled or detectably labeled DNA primers. The labeled DNA primers may be present in lyophilized form or in an appropriate buffer as necessary. One or more containers may contain one or more enzymes or reagents to be utilized in PCR reactions. These enzymes may be present by themselves or in admixtures, in lyophilized form or in appropriate buffers.

Finally, the kit may contain all of the additional elements necessary to carry out the technique of the invention, such as buffers, extraction reagents, enzymes, pipettes, plates, nucleic acids, nucleoside triphosphates, filter paper, gel materials, transfer materials, autoradiography supplies, and the like.

Preferred within the scope of the present invention is a diagnostic kit used in detecting a fungal pathogen, comprising the pair of primers for use in the amplification-based

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detection of a fungal Internal Transcribed Spacer DNA sequence, wherein at least one of said primers is the oligonucleotide primer accoding to the invention.

The examples below show typical experimental protocols that can be used in the isolation of ITS sequences, the selection of suitable primer sequences, the testing of primers for selective and diagnostic efficacy, and the use of such primers for disease and fungal isolate detection. Such examples are provided by way of illustration and not by way of limitation.

DESCRIPTION OF THE FIGURE

FIGURE 1/3,2/3,3/3 Sequence Alignment of the ITS regions from *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium poae*, *Microdochium nivale*, and *Fusarium moniliforme*.

BRIEF DESCRIPTION OF THE SEQUENCES IN THE SEQUENCE LISTING

SEQ ID NO:1 Oligonucleotide Primer ITS1.

SEQ ID NO:2 Oligonucleotide Primer ITS2.

SEQ ID NO:3 Oligonucleotide Primer ITS3.

SEQ ID NO:4 Oligonucleotide Primer ITS4.

SEQ ID NO:5 M13 Universal-20 Primer.

SEQ ID NO:6 Reverse Primer used in Example 2.

SEQ ID NO:7 Oligonucleotide Primer JB605.

SEQ ID NO:8 Oligonucleotide Primer JB606.

SEQ ID NO:9 Oligonucleotide Primer JB607.

SEQ ID NO:10 Oligonucleotide Primer JB609.

SEQ ID NO:11 Oligonucleotide Primer JB610.

SEQ ID NO:12 Oligonucleotide Primer JB611.

SEQ ID NO:13 Oligonucleotide Primer JB612.

SEQ ID NO:14 Oligonucleotide Primer JB613.

SEQ ID NO:15 Oligonucleotide Primer JB614.

SEQ ID NO:16 Oligonucleotide Primer JB578.

SEQ ID NO:17 Oligonucleotide Primer JB571.

SEQ ID NO:18 Oligonucleotide Primer JB572.

SEQ ID NO:19 Consensus DNA sequence of the ITS region PCR-amplified from *Fusarium culmorum* isolates R-5106, R-5126 and R-5146, comprising in the 5' to 3' direction: 3' end of the small subunit rRNA gene, Internal Transcribed Spacer 1, 5.8S rRNA gene, Internal Transcribed Spacer 2, and 5' end of the large subunit rRNA gene.

SEQ ID NO:20 Consensus DNA sequence of the ITS region PCR-amplified from *Fusarium graminearum* isolates R-8417, R-8422 and R-8546, comprising

in the 5' to 3' direction: 3' end of the small subunit rRNA gene, Internal Transcribed Spacer 1, 5.8S rRNA gene, Internal Transcribed Spacer 2, and 5' end of the large subunit rRNA gene.

SEQ ID NO:21 DNA sequence of the ITS region PCR-amplified from *Fusarium moniliforme* isolate 4551, comprising in the 5' to 3' direction: 3' end of the small subunit rRNA gene, Internal Transcribed Spacer 1, 5.8S rRNA gene, Internal Transcribed Spacer 2, and 5' end of the large subunit rRNA gene.

SEQ ID NO:22 Consensus DNA sequence of the ITS region PCR-amplified from *Fusarium poae* isolates T-427, T-534, and T-756, comprising in the 5' to 3' direction: 3' end of the small subunit rRNA gene, Internal Transcribed Spacer 1, 5.8S rRNA gene, Internal Transcribed Spacer 2, and 5' end of the large subunit rRNA gene.

SEQ ID NO:23 Consensus DNA sequence of the ITS region PCR-amplified from *Microdochium nivale* isolates 72, 520, and 18222, comprising in the 5' to 3' direction: 3' end of the small subunit rRNA gene, Internal Transcribed Spacer 1, 5.8S rRNA gene, Internal Transcribed Spacer 2, and 5' end of the large subunit rRNA gene.

SEQ ID NO:24 Consensus DNA sequence of the ITS region PCR-amplified from *Fusarium avenaceum* isolates 64452 and R-4045, comprising in the 5' to 3' direction: 3' end of the small subunit rRNA gene, Internal Transcribed Spacer 1, 5.8S rRNA gene, Internal Transcribed Spacer 2, and 5' end of the large subunit rRNA gene.

EXAMPLES

Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by J. Sambrook, E. F. Fritsch and T. Maniatis, Molecular Cloning: A Laboratory manual, Cold Spring Harbor laboratory, Cold Spring Harbor, NY (1989) and by T.J. Silhavy, M.L. Berman, and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and by Ausubel, F.M. *et al.*, Current Protocols in Molecular Biology, pub. by Greene Publishing Assoc. and Wiley-Interscience (1987).

Example 1: Fungal Isolates and Genomic Fungal DNA Extraction

See Table 1 for a listing of the fungal isolates used and their source. Fungi are grown in 150 ml potato dextrose broth inoculated with mycelial fragments from PDA (Potato Dextrose Agar) cultures. Cultures are incubated on an orbital shaker at 28°C for 7-11 days. Mycelia are pelleted by centrifugation and then ground in liquid nitrogen, and total genomic DNA is extracted using the protocol of Lee and Taylor (1990; In: *PCR Protocols: A Guide to Methods and Applications*; Eds.: Innes *et al.*; pages 282-287).

Table 1: Source of Test Isolates

Isolate	Organism	Origin	Source
69	*Microdochium nivale*	---	Novartis-Basel[1]
72	*Microdochium nivale*	---	Novartis-Basel[1]
92	*Microdochium nivale* var. *majus*	---	Novartis-Basel[1]
93	*Microdochium nivale* var. *majus*	---	Novartis-Basel[1]
520	*Microdochium nivale*	---	Novartis-Basel[1]
18222	*Microdochium nivale*	Scotland	ATCC[2]
T-0427	*Fusarium poae*	Lancaster County, PA USA	P. Nelson[3]
T-0534	*Fusarium poae*	Rock Springs, PA USA	P. Nelson[3]
T-0756	*Fusarium poae*	Unionville, PA USA	P. Nelson[3]
36885	*Fusarium graminearum*	Finland	ATCC[2]
R-8422	*Fusarium graminearum*	Canada	P. Nelson[3]
R-8546	*Fusarium graminearum*	Plevdiv, Bulgaria	P. Nelson[3]
R-8417	*Fusarium graminearum*	Italy	P. Nelson[3]
R-9367	*Fusarium graminearum*	Mercer County, PA, USA	P. Nelson[3]
R-9420	*Fusarium graminearum*	Washington, USA	P. Nelson[3]
R-8637	*Fusarium graminearum*	Settat, Morocco	P. Nelson[3]
4551	*Fusarium moniliforme*	Indiana, USA	L. Castor[4]
13379	*Fusarium roseum*	---	ATCC[2]
64452	*Fusarium avenaceum*	Poland	ATCC[2]
R-4045	*Fusarium avenaceum*	Dubbo, Australia	P. Nelson[3]
R-4941	*Fusarium avenaceum*	Southern Brazil	P. Nelson[3]
R-8547	*Fusarium avenaceum*	Plevdiv, Bulgaria	P. Nelson[3]
R-6554	*Fusarium avenaceum*	Pullman, Washington USA	P. Nelson[3]
62215	*Fusarium culmorum*	Switzerland	ATCC[2]
R-5391	*Fusarium culmorum*	Germany	P. Nelson[3]
R-5126	*Fusarium culmorum*	Minnesota, USA	P. Nelson[3]
R-7770	*Fusarium culmorum*	Poland	P. Nelson[3]
R-5106	*Fusarium culmorum*	Darling Downs, Australia	P. Nelson[3]
R-5146	*Fusarium culmorum*	Finland	P. Nelson[3]
44643	*Pseudocercosporella herpotrichoides* -W type	Germany	ATCC[2]
308	*Pseudocercosporella herpotrichoides* -R type	---	Novartis-Basel[1]
44234	*Ceratobasidium cereale*	Netherlands	ATCC[2]
11404	*Drechslera sorokiniana*	Minnesota, USA	ATCC[2]
60531	*Cladosporium herbarum*	New Zealand	ATCC[2]
38699	*Septoria glycines*	Illinois, USA	ATCC[2]
26517	*Septoria tritici*	Minnesota, USA	ATCC[2]
52476	*Cercospora arachidicola*	Oklahoma, USA	ATCC[2]
26380	*Septoria avenae* f.sp. *triticea*	Minnesota, USA	P. Ueng[5]
24425	*Septoria nodorum*	Montana, USA	ATCC[2]
---	*Rhizoctonia solani* (salvia)	Vero Beach, Florida USA	Novartis-US[6]

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Example 2: Isolation of the Internal Transcribed Spacer (ITS) Regions

Approximately 550-bp long internal transcribed spacer region fragments are PCR amplified from 10 ng of genomic DNA isolated from *F. graminearum* isolates R-8417, R-8422 and R-8546, *F. culmorum* isolates R-5106, R-5126 and R-5146, *F. moniliforme* isolate #4551, *F. poae* isolates T-0427, T-0534 and T-0756, *M. nivale* isolates 520, 72 and 18222, and *F. avenaceum* isolates 64452 and R-4045 using 50 pmol of primers ITS1 (5' TCCGTAGGTGAACCTGCGG-3'; SEQ ID NO:1) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; SEQ ID NO:4). PCRs are performed as described in Example 4. PCR products are purified using Promega's Wizard DNA Clean-up kit (Madison, WI). The DNA sequences of the ITS regions are determined by the dideoxy method using the Applied Biosystems (Foster City, CA) automated sequencer with the primers ITS1 (SEQ ID NO:1), ITS2 (5'-GCTGCGTTCTTCATCGATGC-3'; SEQ ID NO:2), ITS4 (SEQ ID NO:4) and the M13 universal -20 (5'-GTAAAACGACGGCCAGT-3'; SEQ ID NO:5) and Reverse (5'-AACAGCTATGACCATG-3'; SEQ ID NO:6) primers. The ITS primers ITS1, ITS2, ITS3, and ITS4 are detailed in White *et al.* (1990; In: PCR Protocols; Eds.: Innes *et al.* pages 315-322). PCR products from amplifications with *F. moniliforme* isolate #4551, *F. poae* isolates T-0427, T-0534 and T-0756 and *M. nivale* isolates 520, 72 and 18222 are cloned using the Invitrogen Corporation's (San Diego, CA) TA Cloning Kit (part no. K2000-01) using the PCR2.1 cloning vector.

Example 3: DNA Extraction from Wheat

DNA is extracted from wheat using a bulk maceration method. The bulk maceration method is used to isolate DNA from several naturally infected wheat heads or stems from the field to optimize the field sampling method for high throughput analysis.

Bulk Maceration Method:

(1) Place the appropriate number of wheat heads or stems in a Bioreba (Reinach, Switzerland) heavy duty plastic bag (cat#490100). Weigh the plant tissue, plastic bag with leaves minus the tare (weight of the plastic bag).

(2) Add an equal volume (ml) of Muller Extraction Buffer (0.1% w/v Tween-80; 0.04 M Tris-Cl, pH 7.7; 0.15 M NaCl; 0.1% w/v BSA-Pentex fraction V; 0.01% w/v sodium azide; 200 mM EDTA) per weight (g) of wheat tissue. Macerate the tissue using a Bioreba Homex 6 homogenizer set at 70. Grind the leaves until the tissue is fibrous.

(3) Pool the extracts from multiple bags, if used, and vortex well. Aliquote the extraction juice into eppendorf tubes on ice.

(a) Boil 100 µl of the concentrated extract for 5 minutes.

(b) Place the boiled extract on ice.

(c) Make a 1:10 dilution by adding 10 µl from the boiled, concentrated extract to 90 µl of sterile dH_2O.

(d) Store the diluted extracts on ice until ready to use.

Example 4: Polymerase Chain Reaction Amplification

Polymerase chain reactions are performed with the GeneAmp Kit from Perkin-Elmer/Cetus (Norwalk, CT; part no. N808-0009) using 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM Tris-HCl, pH8.3, containing 200 µM of each dTTP, dATP, dCTP, and dGTP, 50 pmol each primer, 2.5 units of *Taq* polymerase and 10 ng of genomic DNA or 1 µl of 1:10 diluted plant extract in a final volume of 50 µl. Reactions are run for 30 - 40 cycles of 15 s at 94°C, 15 s at 50°C - 70°C, and 45 s at 72°C in a Perkin-Elmer/Cetus Model 9600 thermal cycler. The products are analyzed by loading 10 µl of each PCR sample on a 1.0% agarose gel and electrophoresing.

Example 5: Synthesis and Purification of Oligonucleotides

Oligonucleotides (primers) are synthesized by, for example, either Integrated DNA Technologies (Coralville, IA) or Midland Certified Reagent Company (Midland, Texas).

Example 6: Selection of Species-Specific Primers

FIGURE 1 shows an alignment of the sequences of the ITS regions of *F. culmorum*, *F. graminearum*, *F. poae*, *M. nivale*, and *F. moniliforme*. Oligonucleotide primers such as those shown below in Table 2 are synthesized according to Example 5 based on analysis of the aligned sequences. Primers are designed to the regions that contain the greatest

differences in sequence among the fungal species. Primers are also designed to regions highly conserved among the species in attempt to develop genus-specific primers. In addition, the published ribosomal gene-specific primers ITS1, ITS2, ITS3 and ITS4 (White *et al.*, 1990; In: PCR Protocols; Eds.: Innes *et al.* pages 315-322) are synthesized for testing in combination with the primers specific for the ITS regions. Primers targeted to *Fusarium* spp. from WO 95/29260 are also used in combination with the newly designed primers to test for novel specificities.

Table 2: Primers Designed for Fungal Detection

Primer Template	Primer	Primer Sequence
Fusarium spp.[1]	JB605	5'CCAAACCATGTGAACTTACC3' (SEQ ID NO: 7)
M. nivale	JB606	5'GGACTACCTAAACTCTGTT3' (SEQ ID NO: 8)
M. nivale	JB607	5'AGGGATCATTACCGAGTTT3' (SEQ ID NO: 9)
M. nivale	JB609	5'TCCGGCTTGCAGAAGCGAG3' (SEQ ID NO: 10)
M. nivale	JB610	5'GAAGGGTGCGGTTTATGGCT3' (SEQ ID NO: 11)
M. nivale	JB611	5'GCCACCGCCGGTGGAC3' (SEQ ID NO: 12)
M. nivale	JB612	5'GGTGCTGTCTCTCGGGAC3' (SEQ ID NO: 13)
M. nivale	JB613	5'AGTCAATCTGAATCAAACTAAG3' (SEQ ID NO: 14)
M. nivale	JB614	5'CTAAACTCTGTTAATTTTTGTCAA3' (SEQ ID NO: 15)
Fusarium spp.[2]	JB578	5'CCGCGACGATTACCAG3' (SEQ ID NO: 16)
F. gram. + *F. culm.*[3]	JB571	5'TAACGATATGTAAATTACTACGCT3' (SEQ ID NO: 17)
F. gram. + *F. culm.*[3]	JB572	5'AAGTTGGGGTTTAACGGC3' (SEQ ID NO: 18)
18S rDNA	ITS1	5'TCCGTAGGTGAACCTGCGG3' (SEQ ID NO: 1)
5.8S rDNA	ITS2	5'GCTGCGTTCTTCATCGATGC3' (SEQ ID NO: 2)
5.8S rDNA	ITS3	5'GCATCGATGAAGAACGCAGC3' (SEQ ID NO: 3)
25S rDNA	ITS4	5'TCCTCCGCTTATTGATATGC3' (SEQ ID NO: 4)

[1] *Fusarium* spp. includes *F. graminearum, F. culmorum, F. avenaceum, F. poae* and *F. moniliforme.*
[2]*Fusarium* spp. includes *F. graminearum, F. culmorum, F. poae, F. moniliforme* and *F. roseum.*
[3]Primer combination only tested against *F. graminearum, F. culmorum* and *M. nivale.*

Example 7: Determination of Primer Specificity to Purified Fungal Genomic DNA

PCRs are performed according to Example 4 using different primer combinations (Table 3) in an attempt to amplify a single specific fragment. Specific PCR amplification products are produced from primers designed from the ITS regions between the 18S and 25S ribosomal DNA subunits of each fungal strain of interest.

Table 3: ITS-Derived Diagnostic PCR Primers

Primer Specificity	5' Primer	3' Primer	Approximate size of amplified fragment
M. nivale	JB612 (SEQ ID NO:13)	ITS4 (SEQ ID NO:4)	472 bp
M. nivale	JB613 (SEQ ID NO:14)	JB610 (SEQ ID NO:11)	337 bp
M. nivale	JB614 (SEQ ID NO:15)	JB610 (SEQ ID NO:11)	355 bp
M. nivale	JB613 (SEQ ID NO:14)	ITS4 (SEQ ID NO:4)	413 bp
M. nivale	JB614 (SEQ ID NO:15)	ITS4 (SEQ ID NO:4)	431 bp
M. nivale	JB611 (SEQ ID NO:12)	JB609 (SEQ ID NO:10)	346 bp
M. nivale	JB611 (SEQ ID NO:12)	ITS4 (SEQ ID NO:4)	450 bp
M. nivale	ITS1 (SEQ ID NO:1)	JB609 (SEQ ID NO:10)	452 bp
M. nivale	ITS1(SEQ ID NO:1)	JB610 (SEQ ID NO:11)	480 bp
M. nivale	JB605 (SEQ ID NO:7)	JB610 (SEQ ID NO:11)	433 bp
M. nivale	JB606 (SEQ ID NO:8)	JB610 (SEQ ID NO:11)	362 bp
M. nivale	JB607 (SEQ ID NO:9)	JB610 (SEQ ID NO:11)	460 bp
Fusarium spp.[1] + *M. niv*	JB605 (SEQ ID NO:7)	ITS4 (SEQ ID NO:4)	509 bp
Fusarium spp.[2]	JB605 (SEQ ID NO:7)	JB578 (SEQ ID NO:16)	417 bp
F. *gram* . + F. *culm* .[3]	JB605 (SEQ ID NO:7)	JB572 (SEQ ID NO:18)	440 bp
F. *gram* . + F. *culm* .[3]	JB605 (SEQ ID NO:7)	JB571 (SEQ ID NO:17)	400 bp

[1] *Fusarium* spp. includes *F. graminearum, F. culmorum, F. avenaceum, F. poae* and *F. moniliforme.*
[2]*Fusarium* spp. includes *F. graminearum, F. culmorum, F. poae, F. moniliforme* and *F. roseum.*
[3]Primer combination only tested against *F. graminearum, F. culmorum* and *M. nivale.*

Example 8: Determination of Primer Specificity to Plant Tissue Infected with Fungi and Cross-Reactivity with Other Cereal Fungal Pathogens

Total genomic DNA is isolated as described in Example 3 from healthy wheat heads and from wheat heads inoculated with *M. nivale, F. graminearum, F. culmorum,* or *F. avenaceum.* PCRs are performed as described in Example 4 testing primer combinations such as those listed in Table 3 against DNA from the wheat tissue. Purified fungal genomic DNAs are obtained as described in Example 1 and PCR assayed as described in Example 4 using the diagnostic primers. Other fungal DNA species and isolates are tested for the ability of the diagnostic primers to cross-react therewith. The results of representative experiments are as follows:

M. nivale-specific primer combination JB612 (SEQ ID NO: 13) and ITS4 (SEQ ID NO: 4) amplified a 472 bp fragment from DNA from all of the *M. nivale* isolates listed in

Table 1 and from *M. nivale*-infected wheat tissue. This primer combination did not amplify a diagnostic fragment from healthy wheat tissue nor from purified genomic DNA from *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* or *F. moniliforme*. This primer combination also did not amplify a diagnostic fragment from purified genomic DNA isolated from the following common cereal pathogens: *P. herpotrichoides* R- and W-pathotypes, *C. cereale*, *D. sorokiniana*, *C. herbarum*, *S. glycines*, *S. tritici*, *C. arachidicola*, *S. nodorum*, *R. solani* and *S. avenae* f.sp. *triticea*. Similar diagnostic results were obtained with *M. nivale*-specific primer combination JB613 (SEQ ID NO:14) and JB610 (SEQ ID NO:11).

Primer combination JB613 (SEQ ID NO:14) and ITS4 (SEQ ID NO:4) amplified a 413 bp fragment, and primer combination JB614 (SEQ ID NO:15) and ITS4 (SEQ ID NO:4) amplified a 431 bp fragment from DNA from *M. nivale* isolate #520 and from wheat infected with *M. nivale*. These primer combinations did not amplify any fragments from healthy wheat tissue, nor from DNA from *F. graminearum* isolate #R-8422 and *F. culmorum* isolate #R-5391.

The remaining *M. nivale*-specific primer combinations listed in Table 3 amplified a PCR fragment from DNA from *M. nivale* isolate #520 but not from DNA from *F. graminearum* isolate #R-8422 nor *F. culmorum* isolate #R-5391.

Primer combination JB605 (SEQ ID NO:7) and ITS4 (SEQ ID NO:4) amplified a 509 bp fragment from DNA from all of the *M. nivale* isolates listed in Table 1. This primer combination also amplified a 509 bp fragment from DNA from all of the *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* and *F. moniliforme* isolates listed in Table 1. This primer combination did not amplify a diagnostic fragment from purified genomic DNA isolated from the following cereal pathogens: *P. herpotrichoides* R- and W-pathotypes, *C. cereale*, *D. sorokiniana*, *C. herbarum*, *S. glycines*, *S. tritici*, *C. arachidicola*, *S. nodorum*, *R. solani* and *S. avenae* f.sp. *triticea*. Primer combination JB605 (SEQ ID NO:7) and ITS4 (SEQ ID NO:4) also amplified a diagnostic fragment from wheat infected with *Fusarium* spp. but not from healthy wheat.

Primer combinations JB605 (SEQ ID NO:7) and JB571 (SEQ ID NO:17), JB605 (SEQ ID NO:7) and JB572 (SEQ ID NO:18), and JB605 (SEQ ID NO:7) and JB578 (SEQ ID NO:16) amplified 400 bp, 440 bp and 417 bp fragments, respectively, from DNA from *F. graminearum* isolate #R-8422 and *F. culmorum* isolate #R-5391, but not from *M. nivale*

isolate #520. In addition, primer combination JB605 (SEQ ID NO:7) and JB578 (SEQ ID NO:16) amplified a diagnostic fragment from all of the *F. graminearum, F. moniliforme, F. roseum, F. poae* and *F. culmorum* isolates listed in Table 1; however, this primer combination did not amplify from any of the *F. avenaceum* isolates nor *M. nivale* isolates listed in Table 1. Primer combinations JB605 (SEQ ID NO:7) and JB571 (SEQ ID NO:17), JB605 (SEQ ID NO:7) and JB572 (SEQ ID NO:18), and JB605 (SEQ ID NO:7) and JB578 (SEQ ID NO:16) did not amplify a diagnostic fragment from healthy wheat or from purified genomic DNA isolated from the cereal pathogens *P. herpotrichoides* R- and W-pathotypes, *C. cereale, D. sorokiniana, C. herbarum, S. glycines, S. tritici, C. arachidicola, S. nodorum, R. solani* and *S. avenae* f.sp. *triticea*.

While the present invention has been described with reference to specific embodiments thereof, it will be appreciated that numerous variations, modifications, and further embodiments are possible, and accordingly, all such variations, modifications and embodiments are to be regarded as being within the scope of the present invention.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

AUSTRALIAN PATENT OFFICE

EDITORIAL NOTE

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The following sequence pages 1/18 to 18/18 are part of the description.

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(1) GENERAL INFORMATION:

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(iii) NUMBER OF SEQUENCES: 24

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Novartis Corporation Patent Department
(B) STREET: 3054 Cornwallis Road
(C) CITY: Research Triangle Park
(D) STATE: NC
(E) COUNTRY: USA
(F) ZIP: 20779-2257

(v) COMPUTER READABLE FORM:
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(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer ITS1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCCGTAGGTG AACCTGCGG 19

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer ITS2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GCTGCGTTCT TCATCGATGC 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer ITS3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCATCGATGA AGAACGCAGC 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer ITS4"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCCTCCGCTT ATTGATATGC 20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "M13 Universal-20 Primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTAAAACGAC GGCCAGT 17

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Reverse Primer used in Example 2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AACAGCTATG ACCATG 16

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer JB605"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCAAACCATG TGAACTTACC 20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer JB606"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGACTACCTA AACTCTGTT 19

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer JB607"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGGATCATT ACCGAGTTT 19

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer JB609"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TCCGGCTTGC AGAAGCGAG 19

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer JB610"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAAGGGTGCG GTTTATGGCT 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer JB611"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCCACCGCCG GTGGAC 16

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer JB612"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGTGCTGTCT CTCGGGAC 18

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer JB613"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AGTCAATCTG AATCAAACTA AG 22

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer JB614"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTAAACTCTG TTAATTTTTG TCAA 24

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer JB578"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCGCGACGAT TACCAG 16

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer JB571"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TAACGATATG TAAATTACTA CGCT 24

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer JB572"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AAGTTGGGGT TTAACGGC 18

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 504 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Fusarium culmorum
(C) INDIVIDUAL ISOLATE: R-5106, R-5126, and R-5146
(consensus sequence)

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 1..12
(D) OTHER INFORMATION: /note= "3' end of small subunit rRNA gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 13..161
(D) OTHER INFORMATION: /note= "ITS 1"

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 162..318
(D) OTHER INFORMATION: /note= "5.8S rRNA gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 319..472
(D) OTHER INFORMATION: /note= "ITS 2"

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 473..504
(D) OTHER INFORMATION: /note= "5' end of large subunit rRNA gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAGGGATCAT TACCGAGTTT ACTRACTCCC AAACCCCTGT GAACDTACCT TATGTTGCCT 60

CGGCGGATCA GCCCGCGCCC CGTAAAAAGG GACGGCCCGC CGCAGGAACC CTAAACTCTG 120

TTTTTAGTGG AACTTCTGAG TATAAAAAAC AAATAAATCA AAACTTTCAA CAACGGATCT 180

CTTGGTTCTG GCATCGATGA AGAACGCAGC AAAATGCGAT AAGTAATGTG AATTGCAGAA 240

TTCAGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCG CCAGTATTCT GGCGGGCATG 300

CCTGTTCGAG CGTCATTTCA ACCCTCAAGC CCAGCTTGGT GTTGGGAGCT GCAGTCCTGC 360

TGCACTCCCC AAATACATTG GCGGTCACGT CGRAGCTTCC ATAGCGTAGT AATTTACATA 420

TCGTTACTGG TAATCGTCGC GGCYACGCCG TTAAACCCCA ACTTCTGAAT GTTGACCTCG 480

GATCAGGTAG GAATACCCGC TGAA 504

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 503 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Fusarium graminearum
(C) INDIVIDUAL ISOLATE: R-8417, R-8422, and R-8546 (consensus sequence)

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 1..9
(D) OTHER INFORMATION: /note= "3' end of small subunit rRNA gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 10..155
(D) OTHER INFORMATION: /note= "ITS 1"

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 156..312
(D) OTHER INFORMATION: /note= "5.8S rRNA gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 313..466
(D) OTHER INFORMATION: /note= "ITS 2"

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 467..503
(D) OTHER INFORMATION: /note= "5' end of large subunit rRNA gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGATCATTAC CGAGTTTACW SACTCCCAAA CCCCTGTGAA CATACCTTAT GTTGCCTCGG 60

CGGATCAGCC CGCGCCCCGA AAGGGACGGC CCGCCGCAGG AACCCTAAAC TCTGTTTTTA 120

GTGGAACTTC TGAGTATAAA AAACAAATAA ATCAAAACTT TCAACAACGG ATCTCTTGGT 180

KCTGGCATCG ATGAAGAACG CASCRAAATG CGATAAGTAA TGTGWATTGC AGAATTCAGT 240

GAATCAWCGA ATCTTTGAAC GCWSATTGCK MCCRCCAGTA TTCTGGCGGG CATGCCTGTT 300

CGAGCGTCAT TTCAACCCTC AAGCCCAGVT TGGTGTKGGG GARYTGCAGK CCTRYTKCAC 360

TCCCCAAATA ARTTGGCGGT CACGTCGAAC TTCCATAGCG TAGTAAGTTA CACATCGTTA 420

CTGGTAATCG TCGCGGCTAC GCCGTTAAAC CCCAACTTCT GAATGTTGAC CTCGGATCAG 480

GTAGGAATAC CCGCTGAAGG TAA 503

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 545 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Fusarium moniliforme
(C) INDIVIDUAL ISOLATE: 4551

(vii) IMMEDIATE SOURCE:
(B) CLONE: pCRFMON1

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 1..30
(D) OTHER INFORMATION: /note= "3' end of small subunit rRNA gene"

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(B) LOCATION: 31..178
(D) OTHER INFORMATION: /note= "ITS 1"

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 179..335
(D) OTHER INFORMATION: /note= "5.8S rRNA gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 336..488
(D) OTHER INFORMATION: /note= "ITS 2"

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 489..545
(D) OTHER INFORMATION: /note= "5' end of large subunit rRNA gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TCCGTAGGTG AACCTGCGGA GGGATCATTA CCGAGTTTAC AACTCCCAAA CCCCTGTGAA 60

CATACCTTAT GTTGCCTCGG CGGATCAGCC CGCGCCCCGT AAAAAGGGAC GGCCCGCCGC 120

AGGAACCCTA AACTCTGTTT TTAGTGGAAC TTCTGAGTAT AAAAAACAAA TAAATCAAAA 180

CTTTCAACAA CGGATCTCTT GGTTCTGGCA TCGATGAAGA ACGCAGCAAA ATGCGATAAG 240

TAATGTGAAT TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT GCGCCCGCCA 300

GTATTCTGGC GGGCATGCCT GTTCGAGCGT CATTTCAACC CTCAAGCCCA GCTTGGTGTT 360

GGGAGCTGCA GTCCTGCTGC ACTCCCCAAA TACATTGGCG GTCACGTCGA GCTTCCATAG 420

CGTAGTAATT TACACATCGT TACTGGTAAT CGTCGCGGCC ACGCCGTTAA ACCCCAACTT 480

CTGAATGTTG ACCTCGGATC AGGTAGGAAT ACCCGCTGAA CTTAAGCATA TCAATAAGCG 540

GAGGA 545

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 546 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Fusarium poae
(C) INDIVIDUAL ISOLATE: T-427, T-534, and T-756 (consensus sequence)

(vii) IMMEDIATE SOURCE:
(B) CLONE: pCRFpoaeT427(1-2), pCRFpoaeT534(2-2), and pCRFpoaeT756(3-1)

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 1..30
(D) OTHER INFORMATION: /note= "3' end of small subunit rRNA gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 31..180
(D) OTHER INFORMATION: /note= "ITS 1"

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 181..337
(D) OTHER INFORMATION: /note= "5.8S rRNA gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 338..489
(D) OTHER INFORMATION: /note= "ITS 2"

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 490..546

(D) OTHER INFORMATION: /note= "5' end of large subunit rRNA gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TCCGTAGGTG AACCTGCGGA GGGATCATTA CCGAGTTTAC AACTCCCAAA CCCCTGTGAA 60

CATACCTTTA TGTTGCCTCG GCGGATCAGC CCGCGCCCCG TAAAACGGGA CGGCCCGCCG 120

CAGGAAACCC TAAACTCTGT TTTTAGTGGA ACTTCTGAGT ATAAAAAACA AATAAATCAA 180

AACTTTCAAC AACGGATCTC TTGGTTCTGG CATCGATGAA GAACGCAGCA AAATGCGATA 240

AGTAATGTGA ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCGC 300

CAGTATTCTG GCGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCC CAGCTTGGTG 360

TTGGGATCTG TGTGCAAACA CAGTCCCCAA ATTGATTGGC GGTCACGTCG AGCTTCCATA 420

GCGTAGTAAT TTACACATCG TTACTGGTAA TCGTCGCGGC CACGCCGTTA AACCCCAACT 480

TCTGAATGTT GACCTCGGAT CAGGTAGGAA TACCCGCTGA ACTTAAGCAT ATCAATAAGC 540

GGAGGA 546

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 556 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Microdochium nivale
(C) INDIVIDUAL ISOLATE: 72, 520, and 18222 (consensus sequence)

(vii) IMMEDIATE SOURCE:
(B) CLONE: pCRMniv72(5-2), pCRMniv520(4-2), and

pCRMniv18222(6-2)

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 1..30
(D) OTHER INFORMATION: /note= "3' end of small subunit rRNA gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 31..175
(D) OTHER INFORMATION: /note= "ITS 1"

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 176..332
(D) OTHER INFORMATION: /note= "5.8S rRNA gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 333..499
(D) OTHER INFORMATION: /note= "ITS 2"

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 500..556
(D) OTHER INFORMATION: /note= "5' end of large subunit rRNA gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCCGTAGGTG AACCTGCGGA GGGATCATTA CTGAGTTTTT AACTCTCCAA ACCATGTGAA 60

CTTACCACTG TTGCCTCGGT GGATGGTGCT GTCTCTCGGG ACGGTGCCAC CGCCGGTGGA 120

CTACCTAAAC TCTGTTAATT TTTGTCAATC TGAATCAAAC TAAGAAATAA GTTAAAACTT 180

TCAACAACGG ATCTCTTGGT TCTGGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA 240

TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG CCCATTAGTA 300

TTCTAGTGGG CATGCCTGTT CGAGCGTCAT TTCAACCCTT AAGCCTAGCT TAGTGTTGGG 360

AGACTGCCTA ATACGCAGCT CCTCAAAACC AGTGGCGGAG TCGGTTCGTG CTCTGAGCGT 420

AGTAATTTTT TATCTCGCTT CTGCAAGCCG GACTGGCAAC AGCCATAAAC CGCACCCTTC 480

GGGGGCACTT TTTAATGGTT GACCTCGGAT CAGGTAGGAA TACCCGCTGA ACTTAAGCAT 540

ATCAATAAGC GGAGGA 556

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 561 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: Fusarium avenaceum
(C) INDIVIDUAL ISOLATE: 64452 and R-4045 (consensus sequence)

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 1..30
(D) OTHER INFORMATION: /note= "3' end of small subunit rRNA gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 31..181
(D) OTHER INFORMATION: /note= "ITS 1"

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 182..338
(D) OTHER INFORMATION: /note= "5.8S rRNA gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 339..504
(D) OTHER INFORMATION: /note= "ITS 2"

(ix) FEATURE:
(A) NAME/KEY: misc_ feature
(B) LOCATION: 505..561
(D) OTHER INFORMATION: /note= "5' end of large subunit rRNA gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCCGTAGGTG AACCTGCGGA GGGATCATTA CCGAGTTTAC AACTCCCAAA CCCCTGTGAA 60

CATACCTTAA TGTTGCCTCG GCGGATCAGC CCGCGCCCYG TAAAACGGGA CGGCCCGCCA 120

GAGGACCCAA ACTCTAATGT TTCTTATTGT AACTTCTGAG TAAAACAAAC AAATAAATCA 180

AAACTTTCAA CAACGGATCT CTTGGTTCTG GCATCGATGA AGAACGCAGC AAAATGCGAT 240

AAGTAATGTG AATTGCAGAA TTCAGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCG 300

CTGGTATTCC GGCGGGCATG CCTGTTCGAG CGTCATTTCA ACCCTCAAGC CCCCGGGTTT 360

GGTGTTGGGG ATCGGCTCTG CCTTMYGGCG GTGCCGCCCC CGAAATACAT TGGCGGTCTC 420

GCTGCAGCCT CCATTGCGTA GTAGCTAACA CCTCGCAACT GGAACGCGGC GCGGCCATGC 480

CGTAAAACCC CAACTTCTGA ATGTTGACCT CGGATCAGGT AGGAATACCC GCTGAACTTA 540

AGCATATCAA TAAGCGGAGG A 561

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated Internal Transcribed Spacer sequence selected from the group consisting of ITS1 of *Fusarium poae* comprising nucleotides 31-180 of SEQ ID NO:22, ITS2 of *Fusarium poae* comprising nucleotides 338-489 of SEQ ID NO:22, ITS1 of *Microdochium nivale* comprising nucleotides 31-175 of SEQ ID NO:23, ITS2 of *Microdochium nivale* comprising nucleotides 333-499 of SEQ ID NO:23, ITS1 of *Fusarium avenaceum* comprising nucleotides 31-181 of SEQ ID NO:24, and ITS2 of *Fusarium avenaceum* comprising nucleotides 339-504 of SEQ ID NO:24.

2. An oligonucleotide primer for use in identification of a fungal pathogen, wherein said oligonucleotide primer is selected from the group consisting of SEQ ID NO's:7-15.

3. A pair of oligonucleotide primers for use in the amplification-based detection of a fungal Internal Transcribed Spacer DNA sequence, wherein at least one of said primers is the oligonucleotide primer of claim 2.

4. A pair of oligonucleotide primers according to claim 3, wherein said pair is selected from the following primer pairs:

SEQ ID NO:13 and SEQ ID NO:4;
SEQ ID NO:14 and SEQ ID NO:11;
SEQ ID NO:15 and SEQ ID NO:11;
SEQ ID NO:14 and SEQ ID NO:4;
SEQ ID NO:15 and SEQ ID NO:4;
SEQ ID NO12 and SEQ ID NO:10;
SEQ ID NO:12 and SEQ ID NO:4;
SEQ ID NO:1 and SEQ ID NO:10;
SEQ ID NO:1 and SEQ ID NO:11;
SEQ ID NO:7 and SEQ ID NO:11;
SEQ ID NO:8 and SEQ ID NO:11;
SEQ ID NO:9 and SEQ ID NO:11;
SEQ ID NO:7 and SEQ ID NO:4;
SEQ ID NO:7 and SEQ ID NO:16;
SEQ ID NO:7 and SEQ ID NO:18; and
SEQ ID NO:7 and SEQ ID NO:17.

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5. A pair of oligonucleotide primers according to claim 4, wherein said pair of primers are used to detect *Microdochium nivale*, and wherein said pair is selected from the following primer pairs:

SEQ ID NO:13 and SEQ ID NO:4;
SEQ ID NO:14 and SEQ ID NO:11;
SEQ ID NO:15 and SEQ ID NO:11;
SEQ ID NO:14 and SEQ ID NO:4;
SEQ ID NO:15 and SEQ ID NO:4;
SEQ ID NO:12 and SEQ ID NO:10;
SEQ ID NO:12 and SEQ ID NO:4;
SEQ ID NO:1 and SEQ ID NO:10;
SEQ ID NO:1 and SEQ ID NO:11;
SEQ ID NO:7 and SEQ ID NO:11;
SEQ ID NO:8 and SEQ ID NO:11; and
SEQ ID NO:9 and SEQ ID NO:11.

6. A pair of oligonucleotide primers according to claim 4, wherein said pair of primers are used to detect *Microdochium nivale*, *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, and *F. moniliforme*, and wherein said pair comprises SEQ ID NO:7 and SEQ ID NO:4.

7. A pair of oligonucleotide primers according to claim 4, wherein said pair of primers are used to detect *F. graminearum, F. moniliforme, F. roseum, F. poae*, and *F culmorum* and wherein said pair comprises SEQ ID NO:7 and SEQ ID NO:16.

8. A pair of oligonucleotide primers according to claim 4, wherein said pair of primers are used to detect *F. graminearum* and *F. culmorum*, and wherein said pair is selected from the following primer pairs:

SEQ ID NO:7 and SEQ ID NO:18; and
SEQ ID NO:7 and SEQ ID NO:17.

9. A method for the detection of a fungal pathogen selected from the group consisting of *Fusarium poae, Microdochium nivale* and *Fusarium avenaceum*, comprising the steps of:

(a) isolating DNA from a plant leaf infected with said pathogen;

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(b) subjecting said DNA to polymerase chain reaction amplification using at least one primer having at least 10 contiguous nucleotides which are identical to at least 10 contiguous nucleotides of the Internal Transcribed Spacer sequences of nucleotides 31-180 of SEQ ID NO:22, nucleotides 338-489 of SEQ ID NO:22, nucleotides 31-175 of SEQ ID NO:23, nucleotides 333-499 of SEQ ID NO:23, or nucleotides 339-504 of SEQ ID NO:24; and

(c) detecting said fungal pathogen by visualizing the product or products of said polymerase chain reaction amplification.

10. The method of claim 9, wherein said fungal pathogen is *Fusarium poae*.

11. The method of claim 9, wherein said fungal pathogen is Microdochium nivale.

12. The method of claim 9, wherein said fungal pathogen is *Fusarium avenaceum*.

13. An isolated Internal Transcribed Spacer sequence according to claim 1, wherein said Internal Transcribed Spacer sequence is selected from ITS1 and ITS2 of *Fusarium poae*.

14. An isolated Internal Transcribed Spacer sequence according to claim 1, wherein said Internal Transcribed Spacer sequence is selected from ITS1 and ITS2 of *Microdochium nivale*.

15. An isolated Internal Transcribed Spacer sequence according to claim 1, wherein said Internal Transcribed Spacer sequence is selected from ITS1 and ITS2 of *Fusarium avenaceum*.

16. The method of claim 10, wherein said primer has at least 10 contiguous nucleotides which are identical to at least 10 contiguous nucleotides of the Internal Transcribed Spacer sequence of nucleotides 31-180 of SEQ ID NO:22.

17. The method of claim 10, wherein said primer has at least 10 contiguous nucleotides which are identical to at least 10 contiguous nucleotides of the Internal Transcribed Spacer sequence of nucleotides 338-489 of SEQ ID NO:22.

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18. The method of claim 11, wherein said primer has at least 10 contiguous nucleotides which are identical to at least 10 contiguous nucleotides of the Internal Transcribed Spacer sequence of nucleotides 31-175 of SEQ ID NO:23.

19. The method of claim 11, wherein said primer has at least 10 contiguous nucleotides which are identical to at least 10 contiguous nucleotides of the Internal Transcribed Spacer sequence of nucleotides 333-499 of SEQ ID NO:23.

20. The method of claim 12, wherein said primer has at least 10 contiguous nucleotides which are identical to at least 10 contiguous nucleotides of the Internal Transcribed Spacer sequence of nucleotides 339-504 of SEQ ID NO:24.

21. A method for the detection of *Fusarium avenaceum*, comprising the steps of:

(a) isolating DNA from a plant leaf infected with *Fusarium avenaceum*:

(b) subjecting said DNA to polymerase chain reaction amplification using at least one primer having at least 10 contiguous nucleotides identical to at least 10 contiguous nucleotides of nucleotides 31-118 or 138-181 of SEQ ID NO:24; and

(c) detecting *Fusarium avenaceum* by visualizing the product or products of said polymerase chain reaction amplification.

DATED this 20th day of December, 2000
Novartis AG

By DAVIES COLLISON CAVE
Patent Attorneys for the Applicants

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                        10         20         30         40         50         60
Fave.con        TCCGTAGGTGAACCTGCGGAGGGATCATTACCGAGTTTAC~AACTCCCAAACCCCTGTGA  59
Fculm.con       ~~~~~~~~~~~~~~~~~~~~GAGGGATCATTACCGAGTTTACTRACTCCCAAACCCCTGTGA  42
Fgram.con       ~~~~~~~~~~~~~~~~~~~~~~GGATCATTACCGAGTTTACWSACTCCCAAACCCCTGTGA  39
Fpoae.con       TCCGTAGGTGAACCTGCGGAGGGATCATTACCGAGTTTAC~AACTCCCAAACCCCTGTGA  59
Mniv.con        TCCGTAGGTGAACCTGCGGAGGGATCATTACTGAGTTT~TTAACTCTCCAAACCATGTGA  59
PCRFmon1.con    TCCGTAGGTGAACCTGCGGAGGGATCATTACCGAGTTTAC~AACTCCCAAACCCCTGTGA  59

                        70         80         90        100        110        120
Fave.con        ACATACCTTAATGTTGCCTCGGCGGATCAGCCCGCGCCCYGTAAAACGGGACGGCCCGCC 119
Fculm.con       ACDTACCTT~ATGTTGCCTCGGCGGATCAGCCCGCGCCCCGTAAAAAGGGACGGCCCGCC 101
Fgram.con       ACATACCTT~ATGTTGCCTCGGCGGATCAGCCCGCGCCCCG~~~AAAGGGACGGCCCGCC  95
Fpoae.con       ACATACCWTTATGTTGCCTCGGCGGATCAGCCCGCKCCYYGTAAAACGGGACGGCCCGCC 119
Mniv.con        ACTTACCAC~~TGTTGCCTCGGTGGAT~GGTGC~TGTCTCTCGGGACGGTRCCACC~GCC 114
PCRFmon1.con    ACATACCTT~ATGTTGCCTCGGCGGATCAGCCCGCGCCCCGTAAAAAGGGACGGCCCGCC 118

                        130        140        150        160        170        180
Fave.con        AGAGGA~~~CCCAAACTCTAATGTTTCTTATTGTAACTTCTGAGTAAAACAAACAAATAA 176
Fculm.con       GCAGGAA~CCCTAAACTCTG~~~~TTTTTAGTGGAACTTCTGAGTATAAAAAACAAATAA 156
Fgram.con       GCAGGAA~CCCTAAACTCTG~~~~TTTTTAGTGGAACTTCTGAGTATAAAAAACAAATAA 150
Fpoae.con       GCAGGAAACCCTAAACTCTG~~~~TTTTTAGTGGAACTTCTGAGTATAAAAAACAAATAA 175
Mniv.con        GGTGGACTACCTAAACTCTGTTAATTTTTGYCAA~~~~TCTGAATCAAACTAAGAAATAA 170
PCRFmon1.con    GCAGGAA~CCCTAAACTCTG~~~~TTTTTAGTGGAACTTCTGAGTATAAAAAACAAATAA 173

                        190        200        210        220        230        240
Fave.con        ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATG 236
Fculm.con       ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATG 216
Fgram.con       ATCAAAACTTTCAACAACGGATCTCTTGGTKCTGGCATCGATGAAGAACGCASCRAAATG 210
Fpoae.con       ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATG 235
Mniv.con        GTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATG 230
PCRFmon1.con    ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATG 233
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                    250        260        270        280        290        300
                    |          |          |          |          |          |
Fave.con            CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG 296
Fculm.con           CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG 276
Fgram.con           CGATAAGTAATGTGWATTGCAGAATTCAGTGAATCAWCGAATCTTTGAACGCWSATTGCK 270
Fpoae.con           CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG 295
Mniv.con            CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG 290
PCRFmon1.con        CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG 293

                    310        320        330        340        350        360
                    |          |          |          |          |          |
Fave.con            CCCGCTGGTATTCCGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCCCGG 356
Fculm.con           CCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCC~~~A 333
Fgram.con           MCCRCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCC~~~A 327
Fpoae.con           CCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCC~~~A 352
Mniv.con            CCCATTAGTATTCTAGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCTTAAGCCT~~~A 347
PCRFmon1.con        CCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCC~~~A 350

                    370        380        390        400        410        420
                    |          |          |          |          |          |
Fave.con            GTTTGGTGTTGGGGATCGGCTC~~~~TGCCTTMYGGCGGTGCCGCCCCCGAAATACATTG 412
Fculm.con           GCTTGGTGTTGGG~A~~~~~GC~~~~TGCAGTCCTGCTG~~~CACTCCCCAAATACATTG 380
Fgram.con           GVTTGGTGTKGGGGA~~~~~RY~~~~TGCAGKCCTRYTK~~~CACTCCCCAAATAARTTG 375
Fpoae.con           GCTTGGTGTTGGG~A~~~~~TC~~~~TTGYGTKMAWWCR~~~CAKTCCCCAAATTGATTG 399
Mniv.con            GCTTAGTGTTGGGAGACTGCCTAATACGCAGCTCCTCAAAACCAGTGGCGGAGTCGGTTC 407
PCRFmon1.con        GCTTGGTGTTGGG~A~~~~~GC~~~~TGCAGTCCTGCTG~~~CACTCCCCAAATACATTG 397

                    430        440        450        460        470        480
                    |          |          |          |          |          |
Fave.con            GCGGTCTCGCTGCAGCC~TCCATTGCGTAGTAGCTAACACCTCGCAACTGGAACGCGGCG 471
Fculm.con           GCGGTCACGTCGRAGCT~TCCATAGCGTAGTAATTTACATATCGTTACTGGTAATCGTCG 439
Fgram.con           GCGGTCACGTCGAA~CT~TCCATAGCGTAGTAAGTTACACATCGTTACTGGTAATCGTCG 433
Fpoae.con           GCGGTCACGTCGA~GCT~TCCATAGCGTAGTAATTTACACATCGTTACTGGTAATCGTCG 457
Mniv.con            GTGCTCTGAGCGTAGTAATTTTTTATCTCGCT~TCTGCAAGYCG~GACTGGCAACAGCCA 465
PCRFmon1.con        GCGGTCACGTCGA~GCT~TCCATAGCGTAGTAATTTACACATCGTTACTGGTAATCGTCG 455
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                    490        500        510        520        530        540
                     |          |          |          |          |          |
Fave.con        CGG~CCATGCCGTAAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCC 530
Fculm.con       CGG~CYACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCC 498
Fgram.con       CGG~CTACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCC 492
Fpoae.con       CGG~CCACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCC 516
Mniv.con        TAAACCGCACCCTTCGGGGGCACTTTTTAATGGTTGACCTCGGATCAGGTAGGAATACCC 525
PCRFmon1.con    CGG~CCACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCC 514

                    550        560        570        580        590    600
                     |          |          |          |          |      |
Fave.con        GCTGAACTTAAGCATATCAATAAGCGGAGGA                          561
Fculm.con       GCTGAA                                                   504
Fgram.con       GCTGAA~~~~GGTA~~~~~~~~~~~~~~~~~A                         503
Fpoae.con       GCTGAACTTAAGCATATCAATAAGCGGAGGA                          547
Mniv.con        GCTGAACTTAAGCATATCAATAAGCGGAGGA                          556
PCRFmon1.con    GCTGAACTTAAGCATATCAATAAGCGGAGGA                          545
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