PCR Detection of *Fusarium oxysporum* f. sp. *basilici* on Basil

Annalisa Chiocchetti, Dipartimento di Scienze Mediche "A. Avogadro" - Immunologia, Università Piemonte Orientale, Via Solaroli 17, I-28100 Novara, Italy; **Lucia Sciaudone, Fiorenza Durando,** and **Angelo Garibaldi,** Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali - Patologia vegetale, Università di Torino, Via Leonardo da Vinci 44, I-10095 Grugliasco (Torino), Italy; and **Quirico Migheli,** Dipartimento di Protezione delle Piante, Università di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy

ABSTRACT

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Sixty-nine amplified DNA fragments, generated from different isolates of *Fusarium oxysporum* f. sp. *basilici*, were tested for *F. oxysporum* f. sp. *basilici*–specificity in a dot blot assay. One 1,038-bp fragment hybridized to DNA from all *F. oxysporum* f. sp. *basilici* isolates but not to DNA obtained from *F. oxysporum* isolates nonpathogenic to basil or representatives of other formae speciales of *F. oxysporum*, or from isolates of *F. redolens*, *F. tabacinum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *S. minor,* and *Pythium ultimum* obtained from diseased basil. This fragment was cloned and sequenced, and three pairs of *F. oxysporum* f. sp. *basilici*–specific primers were designed, giving rise to amplification products of 943, 382, and 330 bp. A nested PCR assay allowed detection of *F. oxysporum* f. sp. *basilici* in diseased seedlings and in artificially and naturally contaminated seeds. The theoretical detection limit of this system was 10^2 fungal propagules per 100 seeds on artificially contaminated samples, while on naturally contaminated commercial seed lots, 32 propagules per 100 seeds were detected.

Additional keywords: certification, cloning, DNA amplification, Fusarium wilt, RAPD-PCR, sequencing

Wilt and crown rot of sweet basil (*Ocimum basilicum* L.) is incited by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *basilici* (Dzidzariya) Armstr. & Armstr. and represents one of the major phytopathological problems of this increasingly important herb crop (7). Fusarium wilt management in basil normally relies on the integration of various control means, such as soil and substrate disinfestation, cultivation on raised benches, seed dressing with benzimidazoles, and the application of antagonistic *Fusarium* spp. (15–17). The high potential for soil contamination through infested seed (5,6,9,11,13) and airborne propagules (6) makes soil disinfestation inadequate for control of *F. oxysporum* f. sp. *basilici*. The low efficacy of chemical control, limited availability of basil cultivars or ecotypes resistant to Fusarium wilt (18), and inconsistent level of control provided by the commercially available formulations of biocontrol agents (9,16) enhance the need for seed and transplant certification procedures. For this purpose, new techniques are required for the rapid and reliable detection of *F. oxysporum* f. sp. *basilici* and its differentiation from other formae speciales and saprophytic *Fusarium* spp.

The aim of this research was to develop a polymerase chain reaction (PCR)-based method for the fast and unequivocal detection of *F. oxysporum* f. sp. *basilici* from contaminated seed lots and diseased seedlings, avoiding the isolation step on selective medium.

Here we present the selection, cloning, and sequencing of an amplification product generated by the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) (20,21), which has been previously reported as specific for *F. oxysporum* f. sp. *basilici* (2). Three primer sets were designed based on this sequence, allowing for rapid identification of *F. oxysporum* f. sp. *basilici* in infected basil seedlings and contaminated seeds.

MATERIALS AND METHODS

Fungal strains and culture media. Experiments were carried out with 35 isolates of *F. oxysporum* f. sp. *basilici* obtained in 1996 to 1998 from wilted basil, contaminated seed, or infested soil in Italy and Israel (coded FOB 001 through FOB 035), 17 nonpathogenic isolates of *F. oxysporum* obtained from basil seed, flower residues, and soil, nonpathogenic isolates of *F. oxysporum* 233/1, 251/2 (15–17), and single representatives of the formae speciales *canariensis*, *cepae*, *cyclaminis*, *dianthi*, *gladioli*, *lilii*, *lycopersici*, *melonis*, *pisi*, *radicis-lycopersici*, and *tulipae* of *F. oxysporum* (2). Source, geographic origin, and pathogenicity of these isolates on basil have been reported previously (2). Single representatives of *F. redolens*, *F. tabacinum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *S. minor,* and *Pythium ultimum* were isolated from diseased basil seedlings in Albenga (Savona, Italy) during 1996 to 1998. Fungal strains were grown on potato dextrose agar (PDA; Merck, Darmstadt, Germany) and maintained under mineral oil (Sigma, St. Louis, MO) at 12°C.

Genomic DNA extraction. DNA for dot blots and Southern blots was prepared from lyophilized mycelium by a miniprep method described previously (14). A rapid method to obtain DNA for PCR from colonies grown in agar medium (2) was modified as follows: a mycelium-agar plug (50 mg, 1 cm^2) was cut with a sterile scalpel from the fungal colony, crushed in a 1.5-ml tube with 300 µl of 10 mM Tris-HCl, 1 mM EDTA (pH 8) (12), and boiled 10 min. After a 5-min centrifugation at 13,000 rpm to pellet cell debris, 1 µl of the supernatant was used as template in PCR reactions.

Template DNA was extracted from wilted seedlings collected in a previous experiment (2) 14 days after sowing in a substrate infested with each of the 35 *F. oxysporum* f. sp. *basilici* isolates (three seedlings infected by each isolate) and stored at –20°C. To extract total DNA, one seedling was cut into 1-cm sections and placed in a 1.5-ml Eppendorf tube in 50 µl of 10 mM Tris-HCl and 1 mM EDTA (pH 8) solution. The tissue was minced with a scalpel into pieces less than 1 mm^2, and then briefly crushed with a plastic pestle (Eppendorf, Hamburg, Germany), boiled in a water bath for 10 min, and centrifuged at 13,000 rpm for 5 min. One microliter of the supernatant was amplified as described with the primer pair Bik 1 + Bik 4. Control DNA was extracted by following the same protocol from each of three basil seedlings produced in a noninfested substrate. The amplification experiments with DNA extracted from diseased and healthy seedlings were carried out three times (one DNA sample extracted from each seedling in each experiment).

To extract DNA from naturally infested seeds, 100 seeds were placed in 1 ml of extraction buffer (1.4 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, 1% polyvinylpyr-

Corresponding author: Quirico Migheli
E-mail: migheli@ssmain.uniss.it

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rolidone-PVP-, 2% hexadecyltrimethylammonium bromide-CTAB-, pH 8) containing 500 µg of proteinase K per milliliter and gently agitated at 37°C for 15 min. After a 5-min spin at 13,000 rpm, the supernatant was collected, extracted once with phenol:chloroform:isoamylalcohol (25:24:1) and precipitated with exactly 0.4 vol of isopropanol. After a 2-min spin at 13,000 rpm, the pellet was rinsed with 70% ethanol and resuspended in 100 µl of water. Ten microliters of this suspension were used for amplification.

RAPD-PCR conditions. PCR was carried out in 15 µl of reaction mix containing 10 mM Tris-HCl, pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 0.01% (wt/vol) gelatin, 60 µM each of dATP, dCTP, dGTP, and dTTP, 5 pM of primer, about 0.2 ng of template DNA for each isolate, and 0.75 U of Super*Taq* DNA polymerase (HT Biotechnology, Cambridge, UK). Amplification was performed in a Perkin-Elmer Cetus (Norwalk, CT) Gene Amp PCR System 9600 programmed for one cycle of 2.5 min at 94°C, 45 cycles of 30 s at 94°C, 1 min at 36°C, 2 min at 72°C, with no ramping, followed by one cycle of 5 min at 72°C. Gel images were acquired with a Gel Doc 1000 System (Bio-Rad Laboratories, Hercules, CA).

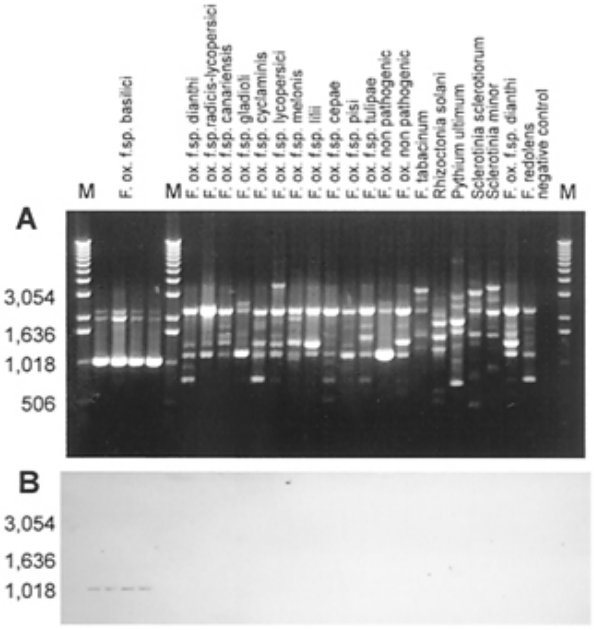

Fig. 1. A, Comparison of random amplified polymorphic DNA (RAPD) patterns obtained with primer OPB-08 from genomic DNA of (from left to right): *Fusarium oxysporum* f. sp. *basilici* isolates FOB 001, FOB 002, FOB 003, and FOB 004, f. sp. *dianthi* isolate FOD1/1, f. sp. *radicis-lycopersici* isolate FORL1, f. sp. *canariensis* isolate Ragusa 2, *gladioli* isolate FOG, f. sp. *cyclaminis* isolate FOCy, f. sp. *lycopersici* isolate FOL race 2, f. sp. *melonis* isolate FOM/R1, f. sp. *lilii* isolate Foli1, f. sp. *cepae* isolate FOCep1, f. sp. *pisi* isolate FOP/R3, f. sp. *tulipae* isolate FOT Nervo, nonpathogenic isolates of *F. oxysporum* 233/1, 251/2, single representatives of *F. tabacinum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *S. minor* isolated from basil seedlings, *F. oxysporum* f. sp. *dianthi* isolate 8/109, *F. redolens*. **B,** Southern blot of RAPD products in **A** probed with the eluted OPB-$08_{1,038}$ amplified fragment. M: molecular weight marker (1-kb DNA ladder, Life Technologies, Gaithersburg, MD). Molecular weight in bp of some marker bands is specified on the left margin.

Table 1. Basil seed lots tested for the presence of *Fusarium oxysporum* f. sp. *basilici* by polymerase chain reaction (PCR) and by isolation on *Fusarium*-selective medium

Lot	Cultivar or ecotype[a]	Geographic origin or distributing company[b]	PCR[c]	CFU/100 seeds (±SD)[d]
1	Malavolti	Genova	Positive	216 ± 31
2	Carnoli	Genova	Positive	32.4 ± 72
3	Ramella autentico	Diano (Imperia)	Negative	1.6 ± 2[e]
4	Filiberto fettine di carne	Diano (Imperia)	Positive	67.2 ± 70
5	Capra Nives	Diano (Imperia)	Positive	176.8 ± 112
6	Armato autentico	Albenga (Savona)	Positive	270.4 ± 208
7	Genovese Gigante S. Remo	Ingegnoli	Positive	446 ± 634
8	Genovese a Foglia media	La Semiorto	Negative	0
9	Genovese sel. DSA	De Corato	Positive	84.8 ± 48
10	Genovese Lot. Ba/09/51	De Corato	Positive	47.2 ± 21
11	Basilico Genovese	Italian Seeds Co.	Positive	719.6 ± 884
12	Fine verde	Carrara	Negative	0
13	Aromatico violetto	Franchi	Positive	175.6 ± 43

[a] For lots 1 to 6 only the local ecotype is available.
[b] Geographic origin of local ecotypes is indicated for lots 1 to 6; commercial lots are produced by different farms in Italy and only the distributing company is indicated.
[c] The test was positive when an *F. oxysporum* f. sp. *basilici*–specific signal of 382 bp was observed after nested amplification with primers Bik 1 + Bik 4 (first round) and Bik 1 + Bik 2 (second round).
[d] The number (±SD) of *F. oxysporum* CFU per 100 seeds was determined in quintuplicate samples.
[e] All *F. oxysporum* colonies isolated from seed lot 3 were nonpathogenic to basil (2).

PCR conditions. The first round of nested PCR was performed in 50 µl of a solution containing 10 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 0.01% (wt/vol) gelatin with the addition of 200 µM of each nucleotide, 0.5 µM of each primer, and 1 µl of crude recombinant *Taq* polymerase prepared from *Escherichia coli* DH5α containing the pTAQ expression vector according to Desai and Pfaffle (3). PCR reactions were performed as described previously (1) with a Perkin-Elmer Cetus Gene Amp PCR System 9600, programmed as follows: 1 cycle for 5 min at 94°C followed by 25 cycles each consisting of a denaturation step at 94°C for 30 s, an extension step at 72°C for 1 min, and annealing temperatures decreasing in the first 10 cycles from 65°C to 55°C for 30 s according to the touchdown program (4).

One microliter of the amplification product was subjected to a second round of nested PCR performed in 25 µl of reaction mix under the same conditions of the first round of PCR except that the extension time was decreased to 30 s. Amplification experiments were always done at least three times.

Cloning and sequencing. Both RAPD-PCR and digested products were fractioned in agarose gel and purified by the Quicksorb kit (Genomed, Research Triangle Park, NC) following the manufacturer's instructions. The OPB-$08_{1,038}$ amplified fragment was ligated into the pGEM-T vector (Promega, Madison, WI) and transformed in *E. coli* XL1 blue (Stratagene, La Jolla, CA). A single *Bam*HI restriction site was found at base 502, and double digestion with either *Apa*I or *Not*I, having a restriction site in the pGEM-T polylinker at the two sides of the insertion site, allowed subcloning into the pBluescript II KS vector (Stratagene). All clones were sequenced by automated sequencing by the Service de Synthèse et d'Analyse at the Department of Recherche en Sciences de la Vie et de la Santé (Université Laval, Québec, Canada) using an ABI 373 DNA sequencer Stretch with XL upgrade (Perkin-Elmer). The fluorescent signals were collected by ABI's Data Collection Software and analyzed by ABI's Sequence Analysis software. The sequence of the BIK 1 clone

was deposited at GenBank (accession no. AF113920).

DNA hybridization. To perform dot blots, 5 µg of total genomic DNA prepared using the miniprep protocol (14) was mixed with 0.5 M NaOH for 5 min and then spotted on a nylon N membrane (Amersham, Little Chalfont, GB) using a vacuum dot blot microfiltration apparatus (Bio-Rad). Each well was washed twice with a 1.5-M NaCl solution and the membrane rinsed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). DNA was fixed 5 min over a 2011 Macrovue transilluminator (LKB, Bromma, Sweden) and the membrane prehybridized for 4 h and hybridized overnight at 65°C.

Chemiluminescent labeling and detection. Probes were eluted from the gel and digoxygenin-labeled with the random primed <DIG> labeling system (Roche, Mannheim, Germany). Hybridization buffer was composed of 5× SSC, 2% wt/vol blocking reagent (Roche), 0.1% *N*-lauroylsarcosine, and 0.02% sodium dodecyl sulfate (SDS). Filters were then washed twice with 2× SSC, 0.1% SDS, and twice with 0.1× SSC, 0.1% SDS at 65°C, 15 min each. Chemiluminescent detection by the nonradioactive <DIG> DNA detection kit (Roche) was performed according to the manufacturer's instructions.

Preparation of spore-seed mixtures. *F. oxysporum* f. sp. *basilici* isolate FOB 001 (2) was grown in 250-ml Erlenmeyer flasks containing 100 ml of potato dextrose broth (PDB; Merck; 24 g liter^{-1}) and yeast extract (Merck; 5 g liter^{-1}) with shaking (150 rpm) at 26°C under constant light. After 7 days, the fungal culture was aseptically filtered through four layers of cheesecloth, and conidia were harvested by centrifugation at 1,500 × *g* for 15 min. After two washes with sterile distilled water, the resulting mixture of macro- and microconidia was counted in a hemacytometer and brought to a final cell density of 10^9 cells ml^{-1}. The conidial suspension was then serially diluted, and 1 ml of each dilution, ranging from 10^9 to 10^1 cells ml^{-1}, was pipetted into 2-ml Eppendorf tubes in five replicates. After a 5-min centrifugation at 13,000 rpm, the pelleted conidia were resuspended in 1 ml of DNA extraction buffer (1.4 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, 1% PVP, 2% CTAB, pH 8, containing 500 µg of proteinase K per milliliter). One hundred seeds of the *F. oxysporum* f. sp. *basilici*–free cv. Fine verde (lot 12; Table 1), were placed in each tube, and DNA extraction from the spore-seed mixture was performed as previously described from naturally infested seeds. The experiment was carried out two times.

CFU determination. The number of CFU of *Fusarium oxysporum* in naturally contaminated seed was determined as follows: five samples of 100 seeds from each lot (Table 1) were placed in 2 ml of sterile Ringer's solution (Merck) and agitated for 30 min. One milliliter of the suspension was then serially diluted from 10^{-1} to 10^{-4}, and 1 ml of each dilution was dispersed into 10 ml of molten *Fusarium oxysporum*–selective medium (10) at 45°C. Colonies were allowed to develop at 30°C for 48 h, and the number of *F. oxysporum* colonies per 100 seeds was determined. The experiment was performed two times.

RESULTS

Evaluation of the RAPD fragment specificity. Sixty-nine RAPD fragments, obtained with 31 different primers, were digoxygenin-labeled and used to probe a dot blot containing genomic DNA of isolates of *F. oxysporum* f. sp. *basilici*, 14 formae speciales of *F. oxysporum,* and different fungi pathogenic on basil.

Only one of the fragments tested, a 1,038-bp RAPD amplicon generated using the OPB-08 primer and named OPB-$08_{1,038}$ (Fig. 1A), gave rise in a dot blot assay to a strong signal with the DNA from the *F. oxysporum* f. sp. *basilici* isolates and a weak or no signal with the other fungal isolates tested (not shown). The OPB-$08_{1,038}$ fragment hybridized with the single 1,038-bp RAPD amplified fragment originating from *F. oxysporum* f. sp. *basilici* DNA, but not with DNA of 20 other fungi (Fig. 1B).

Cloning an *F. oxysporum* f. sp. *basilici*–specific fragment. The OPB-$08_{1,038}$ amplified fragment was cloned in the pGEM-T vector generating clone BIK 1 (Fig. 2). The restriction enzymes *Apa*I/*Bam*HI or *Bam*HI/*Not*I were used to cleave the fragment into two smaller fragments, which were subcloned into two clones coded BIK-AB (594 bp) and BIK-BN (525 bp), respectively.

In order to identify an *F. oxysporum* f. sp. *basilici*–specific region, a dot blot containing genomic DNA from 35 isolates of *F. oxysporum* f. sp. *basilici* and from all the *F. oxysporum* control isolates was probed with digoxygenin-labeled BIK-AB and BIK-BN fragments: both probes hybridized with all *F. oxysporum* f. sp. *basilici* isolate DNAs, but not with the control DNAs (data not shown).

Based on the sequence of the clones, three sets of primers were designed: Bik 1 (5′-ATT CAA GAG CTA AAG GTC C-3′) + Bik 4 (5′-TTT GAC CAA GAT AGA TGC C-3′), Bik 1 + Bik 2 (5′-AAA GGT AGT ATA TCG GAG G-3′), and Bik 3 (5′-GTT CCT ACG GAT AAG ACC-3′) + Bik 4, derived from the sequences of the BIK 1, the BIK-AB, and the BIK-BN clones, respectively (Fig. 2).

Testing primer specificity for *F. oxysporum* f. sp. *basilici*. Expected DNA fragments were amplified from all 35 *F. oxysporum* f. sp. *basilici* isolates using the three primer sets regardless of purification method. No amplification products were detected for DNAs extracted from the non-

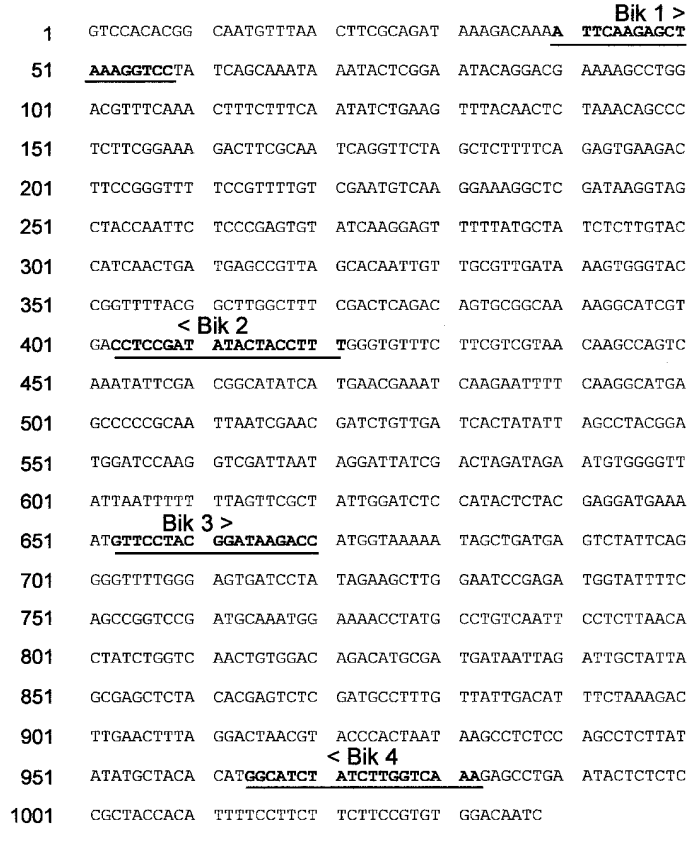

Fig. 2. Sequence and primer selection for clone BIK 1. The GenBank accession number for BIK 1 is AF113920.

pathogenic *F. oxysporum* isolates and the other control templates with any of the primer pairs tested (data not shown). A single DNA fragment was amplified, the size of which depended on the primer pair used: 943 bp (Bik 1 + Bik 4), 382 bp (Bik 1 + Bik 2), or 330 bp (Bik 3 + Bik 4) (Fig. 3). Additionally, DNA extracted from diseased basil seedlings consistently generated an amplification signal of the expected size using the primer set Bik 1 + Bik 4, while no amplification was obtained from DNA extracted from healthy seedlings (data not shown).

PCR assay of naturally infested basil seed and spore-seed mixtures. Total DNA was extracted and PCR performed for 13 seed lots of different origin in Italy (Table 1). An *F. oxysporum* f. sp. *basilici*–PCR specific signal was generated from DNA extracted from 10 of the 13 seed lots tested in nested PCR (first round with the primer set Bik 1 + Bik 4, second round with Bik 1 + Bik 2), confirming the presence of the pathogen. The average number of CFU isolated from these 10 lots on *Fusarium*-selective medium ranged from 32.4 to 719.6 per 100 seeds (Table 1). DNA was extracted from 30 *F. oxysporum*-like colonies chosen randomly from each seed lot and tested in nested PCR. The specific signal expected for *F. oxysporum* f. sp. *basilici* was obtained in all cases. The three seed lots from which no DNA was amplified also did not yield *F. oxysporum* f. sp. *basilici* colonies on *Fusarium*-selective medium. Eight colonies, isolated and tested in a previous experiment (2) from seed lot 3 (Ramella autentico; Table 1), together with seven more isolates obtained from flower residues of the same seed lot, which were morphologically identified as *F. oxysporum*, were all nonpathogenic on the highly sensitive basil cultivar Fine verde (2).

When the nested PCR assay (first round with the primer set Bik 1 + Bik 4, second round with Bik 1 + Bik 2) was conducted with DNA extracted from the spore-seed mixtures, only seed samples spiked with 1 ml of spore suspension ranging from 10^9 to 10^7 spores per milliliter produced an amplification signal of the expected size after the first round PCR. The second round of nested PCR strongly increased the sensitivity of detection, allowing for identification of the pathogen in seed samples mixed with 1 ml of a spore suspension containing 10^2 spores per milliliter (Fig. 4).

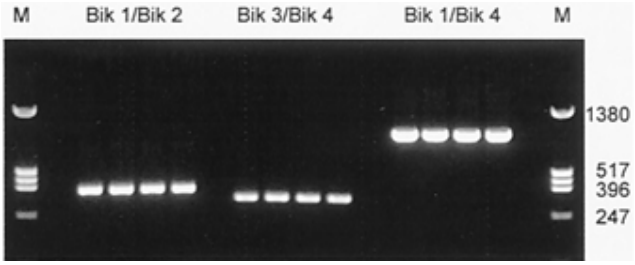

Fig. 3. Polymerase chain reaction (PCR) amplification products obtained from genomic DNA of *Fusarium oxysporum* f. sp. *basilici* isolates FOB 001, FOB 002, FOB 003, and FOB 004 with the primer pairs (from left to right): Bik 1/Bik 2, Bik 3/Bik 4, and Bik 1/Bik 4. M: molecular weight marker (*Hinf*I-digested pVCS vector, Stratagene, La Jolla, CA). Molecular weight in bp of some marker bands is specified on the right margin.

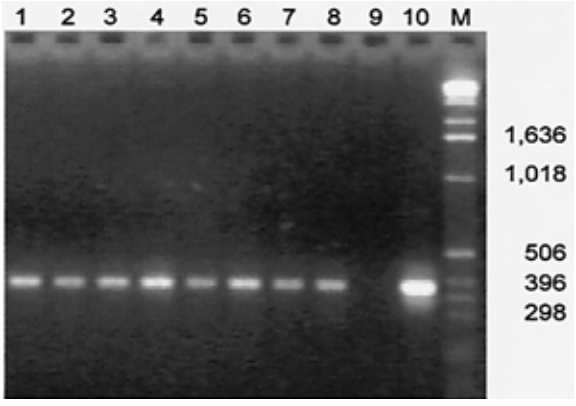

Fig. 4. Nested polymerase chain reaction (PCR) amplification products obtained with primers Bik 1 + Bik 2 from basil seeds mixed to serially titrated spore suspensions of *Fusarium oxysporum* f. sp. *basilici* isolate FOB 001. Lanes 1 to 9: DNA extracted from seed samples mixed to 1 ml of spore suspension containing 10^9 spores to 10^1 spores per milliliter was used as template in the first round of PCR; lane 10: positive control amplification: 10 ng of FOB 001 genomic DNA was used as template in the first round of PCR. M: molecular weight marker (1-kb DNA ladder, Life Technologies, Gaithersburg, MD). Molecular weight in bp of some marker bands is specified on the right margin.

DISCUSSION

The ecological behavior of *F. oxysporum* f. sp. *basilici*, which can be easily disseminated by both airborne and seedborne propagules, requires the development of an integrated management approach to controlling the spread of all the potential sources of inoculum (6,7). All *F. oxysporum* f. sp. *basilici* isolates tested so far from Italy, Israel, and the United States belong to the same vegetative compatibility group (VCG code number 0200, previously assigned to this forma specialis, has been recently changed to 0400 [5,8]). Fast and sensitive diagnostic tools are needed so that primary inoculum vectors, such as seed and transplants, can be screened promptly. The use of *F. oxysporum* f. sp. *basilici*-free certified propagative material will become an essential qualification to worldwide distribution of this crop (7).

In Italy, some seed companies test their product for *F. oxysporum* contamination and sell lots testing negative as certified *F. oxysporum*-free (7). The procedure currently used (isolation on *Fusarium*-selective medium and microscopic observation) does not distinguish among *F. oxysporum* isolates pathogenic and nonpathogenic to basil. As a consequence, lots are discarded if *F. oxysporum* propagules are detected. These seed lots may be contaminated by biotypes of the fungus nonpathogenic to basil. Biocontrol products based on saprophytic *F. oxysporum* (7,9,16) used in propagation may also contaminate seeds. Pathogenicity tests on basil for *F. oxysporum* isolates require 3 to 4 weeks to complete and are not economically feasible.

The PCR-based method we developed provides fast and unequivocal identification of *F. oxysporum* f. sp. *basilici*. This procedure allows sensitive detection directly from wilted plants and seed lots without the isolation of the pathogen.

It has not been determined whether *F. oxysporum* f. sp. *basilici* is an external contaminant or infects seeds internally (13,19), although diseased plants have been obtained from some seed lots after external disinfection (19). Samples from the three seed lots that were negative in the PCR assay were tested on *Fusarium* selective medium or allowed to germinate in disinfected potting substrate. The pathogen was never detected by either method.

By the purification method described here, DNA is extracted only from propagules present on the external surface of the seeds. Complete destruction of seed structure (e.g., by grinding in liquid nitrogen) would make fungal DNA contained in the infecting propagules accessible to primers. In our experience, this approach caused the complete inhibition of PCR reaction. Additional DNA purification steps are therefore needed in order to better ascertain the presence of the pathogen internally.

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LITERATURE CITED

1. Chiocchetti, A., Bernardo, I., Daboussi, M. J., Garibaldi, A., Gullino, M. L., Langin, T., and Migheli, Q. 1999. Detection of *Fusarium oxysporum* f. sp. *dianthi* in carnation tissue by PCR amplification of transposon insertions. Phytopathology 89:1169-1175.
2. Chiocchetti, A., Ghignone, S., Minuto, A., Gullino, M. L., Garibaldi, A., and Migheli, Q. 1999. Identification of *Fusarium oxysporum* f. sp. *basilici* isolated from soil, basil seed, and plants by RAPD analysis. Plant Dis. 83:576-581.
3. Desai, U. J., and Pfaffle, P. K. 1995. Single step purification of a thermostable DNA polymerase expressed in *Escherichia coli*. BioTechniques 19:780-784.
4. Don, R. H., Cox, P. T., Wainwright, B. J., Baker, K., and Mattick, J. S. 1991. 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res. 19:4008.
5. Elmer, W. H., Wick, R. L., and Haviland, P. 1994. Vegetative compatibility among *Fusarium oxysporum* f. sp. *basilicum* isolates recovered from basil seed and infected plants. Plant Dis. 78:789-791.
6. Gamliel, A., Katan, T., Yunis, H., and Katan, J. 1996. Fusarium wilt and crown rot of sweet basil: Involvement of soilborne and airborne inoculum. Phytopathology 86:56-62.
7. Garibaldi, A., Gullino, M. L., and Minuto, G. 1997. Diseases of basil and their management. Plant Dis. 81:124-132.
8. Katan, T., and Di Primo, P. 1999. Current status of vegetative compatibility groups in *Fusarium oxysporum*: Suppl. (1999). Phytoparasitica 27:273-277.
9. Keinath, A. P. 1994. Pathogenicity and host range of *Fusarium oxysporum* from sweet basil and evaluation of disease control methods. Plant Dis. 78:1211-1215.
10. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soils. Rev. Plant Prot. Res. 8:114-124.
11. Kvartskhava, P. A. 1957. On a study of the infectious wilting (fusariosis) in eugenol Basil. Trud. Sukhumsk. Opyt. Sta. Éfiromasl Kul't. 2:101-113 (in *R.A.M.* 38, 613).
12. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
13. Martini, P., and Gullino, M. L. 1991. Trasmissibilità per seme di *Fusarium oxysporum* f. sp. *basilicum*, agente della tracheofusariosi del basilico. Inf. Fitopatol. 41:59-61.
14. Migheli, Q., Friard, O., Del Tedesco, D., Musso, M. R., and Gullino, M. L. 1996. Stability of transformed antagonistic *Fusarium oxysporum* strains *in vitro* and in soil microcosms. Mol. Ecol. 5:641-649.
15. Minuto, A., Garibaldi, A., and Gullino, M. L. 1994. Biological control of Fusarium wilt of basil (*Ocimum basilicum* L.). Proc. BCPC 1994 6D-18:811-816.
16. Minuto, A., Minuto, G., Migheli, Q., Mocioni, M., and Gullino, M. L. 1997. Effect of antagonistic *Fusarium* spp. and of different commercial biofungicide formulations on Fusarium wilt of basil (*Ocimum basilicum* L.). Crop Prot. 16:765-769.
17. Minuto, A., Mocioni, M., and Garibaldi, A. 1995. Preliminary trials on biological control of Fusarium wilt of basil. Acta Hortic. 382:173-177.
18. Reuveni, R., Dudai, N., Putievsky, E., Elmer, W. H., and Wick, R. L. 1997. Evaluation and identification of basil germ plasm for resistance to *Fusarium oxysporum* f. sp. *basilicum*. Plant Dis. 81:1077-1081.
19. Vannacci, G., Cristani, C., Forti, M., Kontoudakis, G., and Gambogi, P. 1999. Seed transmission of *Fusarium oxysporum* f. sp. *basilici* in sweet basil. J. Plant Pathol. 81:47-53.
20. Welsh, J., and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18:7213-7218.
21. Williams, J. G. K., Kubelik, A. R., Livak, K. L., Rafalski, J. A., and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.