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# Characterization of *Colletotrichum gloeosporioides* Isolates from Avocado and Almond Fruits with Molecular and Pathogenicity Tests†

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**One hundred twenty isolates of *Colletotrichum gloeosporioides* from avocado (6 U.S. and 57 Israeli isolates) and almond (57 Israeli isolates) fruits were compared by various molecular methods and a pathogenicity assay in order to determine the genetic diversity and host specificity between and among the different populations. DNA from eight additional U.S. almond anthracnose isolates were also compared. PCR amplification of genomic DNA with four primers produced uniform banding patterns for all the Israeli almond isolates from different geographic locations in Israel. DNAs from the U.S. almond isolates were distinct from DNAs of the Israeli isolates. In contrast, the avocado isolates from Israel and the United States were more diverse, with numerous arbitrarily primed-PCR phenotypes being observed. *Hae*III digestion patterns of A+T-rich DNA distinguished between the almond and avocado isolates. Southern hybridization of the repetitive nuclear-DNA element GcpR1 to *Pst*I-digested genomic DNA of almond and avocado isolates revealed no polymorphic fragments among the almond isolates, whereas polymorphic fragments were observed among the avocado isolates. Amplification and subsequent restriction enzyme digestion of the internal transcribed spacer 4 and 5 regions between the small and large nuclear subunits of DNA encoding rRNA failed to distinguish between *C. gloeosporioides* isolates from a diverse host range. In artificial inoculations, avocado isolates produced various lesions on avocado and almond fruits, whereas the almond isolates infected both fruits at a lower rate.**

The filamentous fungal plant pathogen *Colletotrichum gloeosporioides* (Penzig) Penzig et Sacc. causes anthracnose disease on various temperate, subtropical, and tropical fruits worldwide (1, 2, 6, 8, 32). Avocado and almond anthracnose are major diseases in Israel (3, 27). *C. gloeosporioides* is a postharvest pathogen of avocado that attacks fruit during growth in the orchard. Infections remain quiescent until the fruit ripens, causing symptom development and substantial decay losses during storage and marketing (20, 21). In contrast, almond anthracnose is a disease of young fruit with symptoms that develop during the spring in the orchard. The pathogen causes severe yield losses, and many infected almond orchards have been uprooted in Israel (27, 30). Isolates of *C. gloeosporioides* from avocados and almonds have different optimal growth temperatures, i.e., 20 to 22°C for almond isolates and 26 to 28°C for avocado isolates (28). In addition, these isolates also differ in their susceptibility to benzimidazole fungicides. Almond isolates are insensitive to benzimidazoles in contrast to the sensitivity of avocado isolates (27, 28). Although avocado and almond groves are cultivated in close proximity in certain areas in Israel, where anthracnose occurs in both crops during the spring season, it is not evident whether isolates of avocado infect almonds and vice versa.

In recent years much work has been done to determine the genetic complexity of the *C. gloeosporioides* isolates that infect subtropical and tropical fruits. A variety of molecular approaches have been used, since morphotaxonomic criteria are not accurate enough to discriminate between species. The GcpR1 repetitive nuclear-DNA element from *Colletotrichum lindemuthianum* (24) and A+T-rich DNA have recently been used for grouping various isolates of *C. gloeosporioides* from strawberries (10). Another repetitive sequence comprising human minisatellite DNA has been useful for separating morphologically indistinguishable isolates of *C. gloeosporioides* from *Stylosanthes* spp., a tropical pasture legume (4). Arbitrarily primed-PCR (ap-PCR) analysis has been applied to differentiate between isolates of *C. gloeosporioides* from strawberries (11, 29) and a variety of tropical fruits including avocados, mangoes, and papayas (18). Likewise, polymorphisms in DNA encoding ribosomal RNA (rDNA) and mitochondrial DNA (mtDNA) have been used to assess the extent of variability within populations of *C. gloeosporioides* that infect tropical fruit (1, 16). It appears from the above-mentioned studies that isolates of *C. gloeosporioides* from avocados vary in rDNA and mtDNA banding patterns, whereas mango isolates have uniform rDNA banding patterns and very similar mtDNA, irrespective of their geographic origin (16, 18).

In this study we used a *C. gloeosporioides* collection comprising 63 isolates from avocado fruit, 57 isolates from almond fruit, two isolates from apples, and one from strawberries. Additional species included eight isolates of *Colletotrichum* spp. from the United States that caused almond anthracnose, one isolate of *Colletotrichum fragariae*, and one of *Colletotrichum acutatum*, both from strawberries. Almond and avocado isolates were compared by four independent molecular methods: (i) ap-PCR amplification of genomic DNA, (ii) A+T-rich-DNA patterns generated by *Hae*III digestion associated with the mitochondrial genome, (iii) nuclear-DNA patterns identified by the GcpR1 repetitive element, and (iv) restriction digest analysis of PCR-amplified rDNA. Pathogenicity was assayed by inoculating detached avocado and almond fruits with *C. gloeosporioides* isolates from both hosts.

By using molecular markers to analyze isolates of *C. gloeosporioides* obtained from almond and avocado fruits, we aimed

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TABLE 1. Isolates of *Colletotrichum* spp. used in this study

| Species | Representative isolate | Origin | Host plant |
|---|---|---|---|
| *C. fragariae* | CF-63-1 | Mississippi | Strawberry |
| *C. acutatum* | CA-0037-1 | California | Strawberry |
| *C. gloeosporioides* | CG-231 | Florida | Strawberry |
| *C. gloeosporioides* | APL-2 | South Carolina | Apple |
| *C. gloeosporioides* | APL-7 | North Carolina | Apple |
| *C. gloeosporioides* | MD-22 | Florida | Avocado |
| *C. gloeosporioides* | MD-23 | Florida | Avocado |
| *C. gloeosporioides* | MD-24 | Florida | Avocado |
| *C. gloeosporioides* | MD-25 | Florida | Avocado |
| *C. gloeosporioides* | MD-26 | Florida | Avocado |
| *C. gloeosporioides* | MD-27 | Florida | Avocado |
| *C. gloeosporioides* | AVO-AS-1[a] | Israel | Avocado |
| *C. gloeosporioides* | AVO-AS-9[b] | Israel | Avocado |
| *C. gloeosporioides* | AVO-32-3A[c] | Israel | Avocado |
| *C. gloeosporioides* | AVO-35-5C[d] | Israel | Avocado |
| *C. gloeosporioides* | AVO-37[e] | Israel | Avocado |
| *C. gloeosporioides* | AVO-37-5B[f] | Israel | Avocado |
| *C. gloeosporioides* | AVO-42-7E[f] | Israel | Avocado |
| *C. gloeosporioides* | AVO-47[b] | Israel | Avocado |
| *C. gloeosporioides* | AVO-CG-14[g] | Israel | Avocado |
| *C. gloeosporioides* | AVO-B.Y.-2 | Israel | Avocado |
| *Colletotrichum* sp. | ALM-1[h] | California | Almond |
| *C. gloeosporioides* | ALM-BZR-1C[i] | Southern Israel | Almond |
| *C. gloeosporioides* | ALM-GOZ-93[j] | Northern Israel | Almond |
| *C. gloeosporioides* | ALM-GVA-6A[j] | Northern Israel | Almond |
| *C. gloeosporioides* | ALM-GZT-1F[j] | Northern Israel | Almond |
| *C. gloeosporioides* | ALM-KN-17Q[j] | Northern Israel | Almond |
| *C. gloeosporioides* | ALM-KSH-10[j] | Northern Israel | Almond |
| *C. gloeosporioides* | ALM-KYZ-10[j] | Northern Israel | Almond |
| *C. gloeosporioides* | ALM-NRB-1D[i] | Southern Israel | Almond |
| *C. gloeosporioides* | ALM-NRB-1E[i] | Southern Israel | Almond |

[a] Other representative isolates were AVO-AS-2, -AS-3, -AS-4, 31-1A, -31-5B, and -36-5C.
[b] Other representative isolates were AVO-AS-5, -AS-6, -AS-7, -AS-8, -31, -32-4D, and -47.
[c] Other representative isolates were AVO-31-4B and -33-4C.
[d] Other representative isolates were AVO-35-4A and -CG-14.
[e] Other representative isolates were AVO-33-1A, -33-2C, -33-5K, -36-1C, -36-2A, -36-6C, -37-2A, -37-4B, -37-4D, -38-1D, -38-2C, -38-3D, -39-2C, -39-4P, -40-2U, -40-3M, -40-5A, -40-6D, -40-7T, -48-2A, and -48-2B.
[f] Other representative isolates were AVO-34-2A, -34-4A, -43-1A, -43-7L, -44-3A, -44-1D, -44-2Q, -46-4P, -47-1C, -47-3L, and -48-7B.
[g] Received from D. Prusky (19).
[h] Other isolates were ALM-3, -4, -5, -6, -7, -11, and -12.
[i] Other *C. gloeosporioides* isolates of identical genotype from almond were ALM-BZR-2P, -BZR-2W, -NA-1Q, -NA-2R, -NA-3A, -NA-3D, -NA-7Q, -NRB-20K, -NRB-23E, -NRB-24D, -NRB-27F, -NRB-29B, -NRB-31C, -NRB-32E, -NRB-34L, -NRB-37B, and -NRB-42N.
[j] Other *C. gloeosporioides* isolates of identical genotype from almond were ALM-GOZ-1A, -GOZ-31C, -GOZ-33H, -GOZ-35K, -GOZ-37R, -GOZ-38D, -GOZ-39A, -GOZ-40L, -GOZ-42B, -GZT-1E, -GZT-5B, -IKS-3D, -IKS-4F, -IKS-5L, -IKS-7Q, -IKS-8C, -IKS-9B, -IKS-10N, -IKS-12R, -KN-2P, -KN-3T, -KYZ-6T, -KYZ-6W, -ME-1, -ME-2, -ME-3, -ME-4, -ME-5, -ME-6, -YAF-3E, and -YAF-4E.

to determine whether anthracnose of these two hosts is caused by the same *C. gloeosporioides* population in Israel. In addition, the pathogenicity of isolates from each host was assayed on fruit from both host plants.

## MATERIALS AND METHODS

**Fungal cultures and growth conditions.** The monoconidial *Colletotrichum* cultures used in this study are listed in Table 1. The Israeli *C. gloeosporioides* isolates from avocados originated from infected fruit collected from packinghouses and supermarkets in Israel during the years 1993 to 1995. The U.S. *C. gloeosporioides* material from avocados was kindly provided by J. C. Correll, University of Arkansas. Biological material of *Colletotrichum* spp. responsible for almond anthracnose in the United States was kindly provided by B. Teviotdale, University of California, Parlier. In Israel, cultures of *C. gloeosporioides* from almonds were isolated during the years 1993 to 1995 from infected fruit from groves situated in northern and southern regions of the country. *C. gloeosporioides* isolates 0037-1 from strawberries (10, 11, 29), APL-2 and APL-7 from apples (2), *C. fragariae* isolate CF-63-1 (11, 29), and *C. acutatum* isolate 0037-1 (11) were previously characterized, and DNAs from these isolates are used as a reference in this study. All fungi were cultured in the dark on modified Mathur's medium (0.1% yeast extract, 0.1% Bacto Peptone, 1% sucrose, 0.25% $MgSO_4 \cdot 7H_2O$, 0.27% $KH_2PO_4$, 1.2% agar supplemented with 25 mg of ampicillin in 1 liter of sterile distilled water) (31) at 25°C for the avocado isolates or at 20°C for the almond isolates. Liquid cultures comprising 100 ml of Mathur's medium devoid of agar in 250-ml Erlenmeyer flasks were inoculated with five mycelial disks derived from colony margins. The cultures were agitated for 5 to 6 days on a rotary shaker at 150 rpm and maintained at 25 and 20°C for avocado and almond isolates, respectively. Twelve hours before the mycelia were harvested, the cultures were fragmented by blending for 10 s at 24,000 rpm with a tissue homogenizer (Ultra-Turrax T25; Janke & Kunkel, IKA Labortechnik, Staufen, Germany).

**Isolation and purification of fungal DNA.** Mycelia from 100-ml liquid cultures were collected by vacuum filtration and lyophilized until dry. DNA was extracted and purified as previously described (10, 22). The DNA was suspended in 0.5 ml of TE (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) buffer to an approximate concentration of 200 to 500 μg/ml and diluted to a concentration of 10 to 100 ng/ml for ap-PCR.

**PCR amplification.** For ap-PCR, primers were derived from the following minisatellite or repeat sequences as previously described: CAGCAGCAGCAG CAG (24), TGTCTGTCTGTCTGTC (10), GACACGACACGACAC (14), and GACAGACAGACAGACA (33). In the text, these primers have been designated $(CAG)_5$, $(TGTC)_4$, $(GACAC)_3$, and $(GACA)_4$, respectively. PCR primers for amplification of the internal transcribed spacer 4 (TCCTCCGCTTATTGAT ATGC) and 5 (GGAAGTAAAAGTCGTAACAAGG) regions between the small and large nuclear rDNA, including the 5.8S rDNA, were as described previously (34). PCRs were performed in a total volume of 20 μl, containing 10 to 100 ng of genomic DNA; 50 mM KCl; 10 mM Tris-HCl; 0.2 mM (each) dATP, dCTP, dGTP, and dTTP; 1.5 mM $MgCl_2$; 1 U of *Taq* DNA polymerase (Promega); and 1 μM primer. The reaction mixtures were incubated in a Hybaid

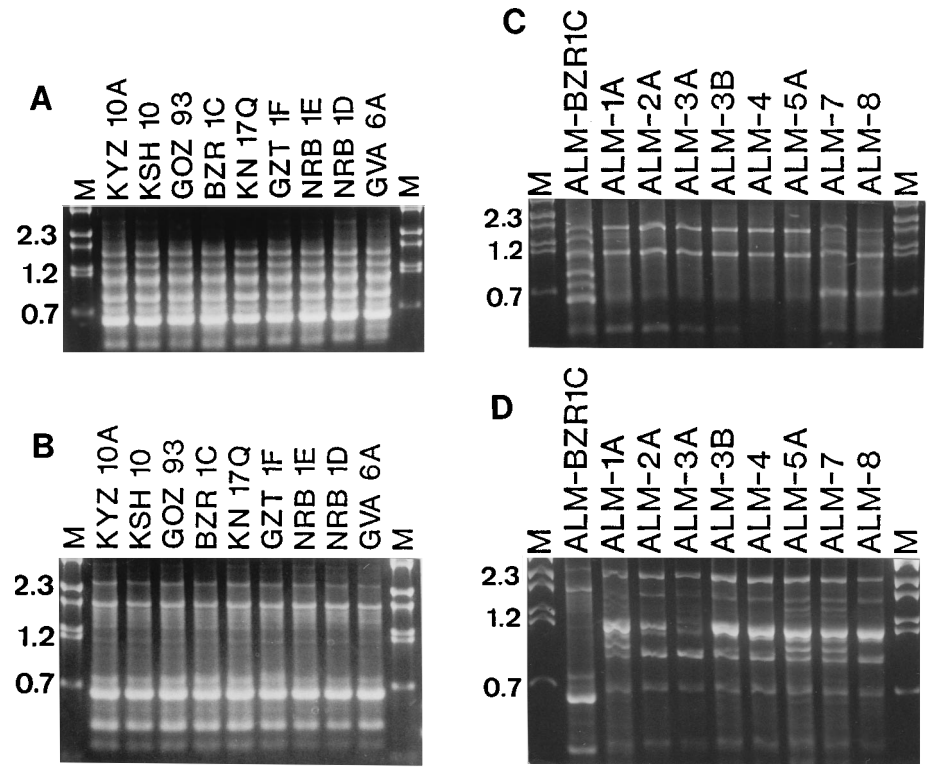


FIG. 1. Band patterns of ap-PCR-amplified genomic DNA from Israeli almond isolates of *C. gloeosporioides* obtained by using primers $(CAG)_5$ (A) and $(GACAC)_3$ (B). Similarly, band patterns of genomic DNA from a representative Israeli isolate (ALM-BZR1C) and from U.S. *Colletotrichum* isolates from almonds were ap-PCR amplified by using primers $(CAG)_5$ (C) and $(GACAC)_3$ (D). Lanes M contain DNA markers, with sizes (in kilobases) on the left.

thermocycler (Hybaid Ltd., Teddington, Middlesex, United Kingdom) starting with 5 min of denaturation at 95°C. For ap-PCR, this was followed by 30 cycles consisting of 30 s at 95°C, 30 s at either 60°C [for $(CAG)_5$] or 48°C [for $(GACA)_4$, $(GACAC)_3$, and $(TGTC)_4$], and 1.5 min at 72°C. For rDNA PCRs, this was followed by 40 cycles consisting of 30 s at 95°C, 30 s at 50°C, and 1.5 min at 72°C. Amplification products were separated in agarose gels (1.5%, 15 by 10 cm) in TAE buffer (26) that were electrophoresed at 80 V for 2 h.

**A+T-rich- and nuclear-DNA analyses.** A+T-rich DNA was analyzed by *Hae*III digestion of total genomic DNA, which cleaves DNA at GGCC sites and leaves A and T sequences intact. A+T-rich sequences are cleaved less frequently, whereas *Hae*III digests nuclear DNA to fragments less than 2 kb in size (10, 12). A+T-rich DNA is partially associated with the mitochondrial genome although contaminating nuclear A+T-rich DNA may also be present.

Nuclear DNA was characterized by digestion of total genomic DNA with *Pst*I and subsequent Southern hybridization to the GcpR1 repetitive DNA from *C. lindemuthianum* (24). GcpR1 hybridizes to nuclear DNA alone and not to A+T-rich DNA (10, 24).

The procedures used for restriction enzyme digestion with *Hae*III and *Pst*I, agarose gel electrophoresis, and Southern hybridization were similar to those previously described (10, 26). $^{32}$P-radiolabeled DNA probes of GcpR1 were made by the random priming method (9) and hybridized as previously described (10). Membranes were autoradiographed by exposure to X-O AR X-ray films (Kodak) at −70°C.

**Restriction enzyme digestion of amplified rDNA.** PCR-amplified rDNA products from representative isolates of *Colletotrichum* spp. were extracted from agarose gels with the Jetsorb kit (Genomed GmbH, Bad Oeynhausen, Germany). Recovered DNA was digested with the restriction enzymes *Bam*HI, *Eco*RI, *Hae*III, *Hin*dIII, *Pst*I, *Sau*3A, and *Stu*I and separated on agarose gels.

**Pathogenicity assays.** Spot inoculations of both avocado and almond fruits were carried out by placing 5-μl water suspensions of conidia ($10^6$/ml) on the fruit surface. From a group of 30 isolates of *Colletotrichum* spp. (Table 1), six representative avocado isolates, including the most and least aggressive isolates, and five almond isolates from different locations were chosen for the pathogenicity assay. Avocado spot inoculations were done at three points along the longitudinal axis of the fruit, whereas each almond fruit was spot inoculated once. Ten fruits were inoculated per isolate. The fruits were maintained under high-humidity conditions for 24 h at 20°C. Lesion diameters of decayed avocado and almond fruits were assessed as previously described (19, 30).

**Representative data and analyses.** In this work, all experiments were repeated at least twice with identical results being reproduced. Percent similarity of ap-PCR-amplified products among avocado isolates was calculated by pairwise comparisons with the SIMQUAL program of NTSYS-pc, version 1.8 (25). The bands chosen for analyses by SIMQUAL are indicated in Fig. 2 by white tick marks. It should be noted, however, that comigrating DNA bands do not necessarily denote sequence homology. The significance of pathogenicity data was determined by using a *t* test.

## RESULTS

**Comparison of avocado and almond *C. gloeosporioides* isolates by ap-PCR.** Genomic DNA from 65 almond and 63 avocado *C. gloeosporioides* isolates was amplified by ap-PCR with primers $(CAG)_5$, $(GACAC)_3$, $(TGTC)_4$, and $(GACA)_4$. No polymorphisms were observed among the Israeli almond isolates. The banding patterns obtained by using primers $(CAG)_5$ and $(GACAC)_3$ (Fig. 1A and B, respectively) and primers $(TGTC)_4$ and $(GACA)_4$ (data not shown) were identical for nine representative almond isolates from different geographic locations. DNA from eight isolates of *Colletotrichum* spp. that caused anthracnose of almond fruit in the United States was compared with DNA from representative Israeli almond isolates by ap-PCR. No identity was observed between Israeli and U.S. isolates by using primers $(CAG)_5$ and $(GACAC)_3$ (Fig. 1C and D, respectively) and primers $(TGTC)_4$ and $(GACA)_4$ (data not shown).

Ap-PCR performed on 57 of the Israeli avocado isolates with the four primers mentioned above led to the identification of eight distinct phenotypes (Fig. 2). A representative of each phenotype, considered to be clonal, was used for further molecular analyses. Isolates of *C. gloeosporioides* from avocados were more complex than isolates from the almond population. PCR-amplified products from the eight representative isolates were compared by using primers $(CAG)_5$ and $(GACAC)_3$ (Fig.

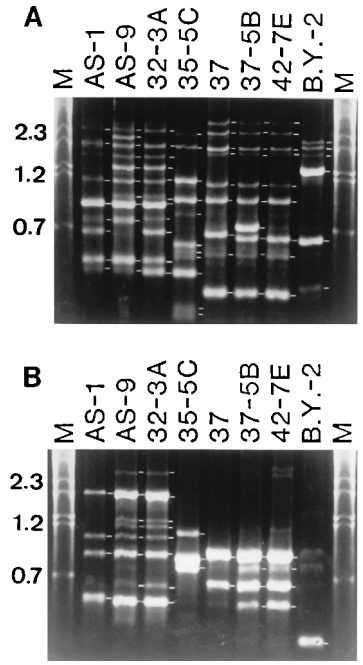


FIG. 2. Band patterns of ap-PCR-amplified genomic DNA from Israeli avocado isolates of *C. gloeosporioides* obtained by using primers $(CAG)_5$ (A) and $(GACAC)_3$ (B). White tick marks indicate the bands chosen to determine percent similarity according to NTSYS-pc. Lanes M contain DNA markers, with sizes (in kilobases) on the left.

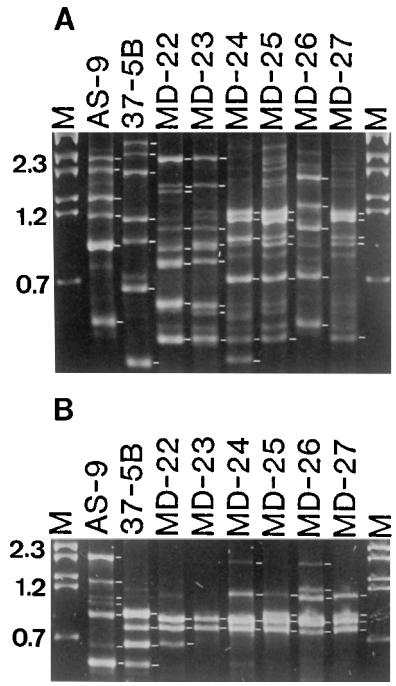


FIG. 3. Band patterns of ap-PCR-amplified genomic DNA from representative Israeli and U.S. avocado isolates of *C. gloeosporioides* obtained by using primers $(CAG)_5$ (A) and $(GACAC)_3$ (B). White tick marks indicate the bands chosen to determine percent similarity according to NTSYS-pc. Lanes M contain DNA markers, with sizes (in kilobases) on the left.

2A and B, respectively). Two main groups of avocado isolates, each including three representative isolates, were compared quantitatively by using the NTSYS-pc analysis system. A similarity of 70.5 to 90% was calculated for the representative avocado isolates AVO-AS-1, -AS-9, and -32-3A belonging to group 1 (Table 2). In group 2, isolates AVO-37, -37-5B, and -42-7E had 78 to 97.5% similarity. However, comparison of isolates between these two groups showed a 0 to 18.5% similarity (Table 2). The results obtained by using all the primers indicated that isolates AVO-35-5C and -B.Y.-2 had amplified bands which were distinctly different from those of the other isolates, and these isolates were only 0 to 10% similar to other avocado isolates (Fig. 2A and B; Table 2).

Six additional U.S. isolates of *C. gloeosporioides* from avocados were compared by ap-PCR with two representative Israeli isolates, AS-9 and 37-5B. PCR-amplified products from the eight isolates were compared by using primers $(CAG)_5$ and $(GACAC)_3$ (Fig. 3A and B, respectively). The six U.S. isolates evidenced a low level of identity with the representative Israeli isolates, 6.7 to 20.8% similarity (Fig. 3A and B; Table 3). Distinct differences also existed among the U.S. isolates, with similarity ranging from 31.3 to 95.5% (Table 3).

**Comparison of avocado and almond *C. gloeosporioides* isolates by A+T-rich-DNA analysis.** A+T-rich DNA, associated with mtDNA, was analyzed by digesting genomic DNA from representative *C. gloeosporioides* isolates from avocados and almonds with *Hae*III. Band patterns of isolates from avocados were distinct from those of the almond isolates (Fig. 4). Polymorphisms were observed among the DNAs of six avocado isolates (AVO-AS-1, -AS-9, -32-3A, -35-5C, -37, and -B.Y.-2),

TABLE 2. Matrix of similarity of avocado *C. gloeosporioides* isolates from Israel based on ap-PCR[a]

| Isolate | Similarity (%) to isolate: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS-1 | AS-9 | 32-3A | 35-5C | 37 | 37-5B | 42-7E | B.Y.-2 |
| AS-1 | | | | | | | | |
| AS-9 | 70.5 | | | | | | | |
| 32-3A | 77.5 | 90.0 | | | | | | |
| 35-5C | 11.0 | 10.0 | 10.0 | | | | | |
| 37 | 11.0 | 5.0 | 10.0 | 10.0 | | | | |
| 37-5B | 0.0 | 18.5 | 18.5 | 10.0 | 81.0 | | | |
| 42-7E | 0.0 | 18.5 | 18.5 | 10.0 | 78.0 | 97.5 | | |
| B.Y.-2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |

[a] Percentage similarity is the average calculated from pairwise comparisons done by using SIMQUAL of NTSYS among representative avocado isolates with primers $(CAG)_5$ and $(GACAC)_3$ from Fig. 2 and primers $(TGTC)_4$ and $(GACA)_4$ (data not shown). Standard error is below 12 for PCR data.

TABLE 3. Matrix of similarity of avocado *C. gloeosporioides* isolates from Israel and the United States based on ap-PCR[a]

| Isolate | Similarity (%) to isolate: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS-9 | 37-5B | MD-22 | MD-23 | MD-24 | MD-25 | MD-26 | MD-27 |
| AS-9 | | | | | | | | |
| 37-5B | 18.5 | | | | | | | |
| MD-22 | 7.2 | 14.3 | | | | | | |
| MD-23 | 6.7 | 16.7 | 69.4 | | | | | |
| MD-24 | 7.7 | 20.2 | 35.7 | 40.0 | | | | |
| MD-25 | 7.7 | 20.2 | 42.9 | 40.0 | 79.2 | | | |
| MD-26 | 8.3 | 11.1 | 33.3 | 35.7 | 42.4 | 31.3 | | |
| MD-27 | 8.3 | 20.8 | 42.9 | 40.5 | 73.9 | 95.5 | 40.0 | |

[a] Percentage similarity is the average calculated from pairwise comparisons done by using SIMQUAL of NTSYS among representative avocado isolates with primers $(CAG)_5$ and $(GACAC)_3$ from Fig. 3 and primers $(TGTC)_4$ and $(GACA)_4$ (data not shown). Standard error is below 15 for PCR data.

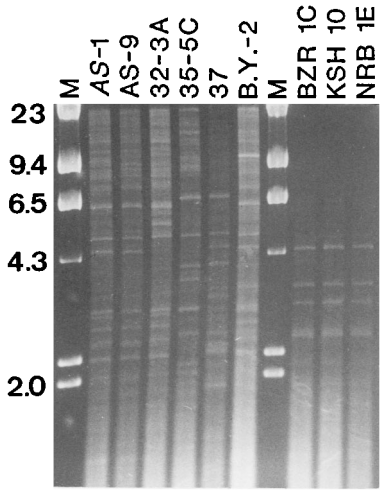


FIG. 4. Band patterns of genomic DNA from isolates of *C. gloeosporioides* from avocado fruits (AS-1, AS-9, 32-3A, 35-5C, 37, and B.Y.-2) and almond fruits (BZR 1C, KSH 10, and NRB 1E) digested with *Hae*III for A+T-rich-DNA analysis. DNA was electrophoresed until the major G+C-rich fragments were eluted from the gel. Lanes M contain DNA markers, with sizes (in kilobases) on the left.

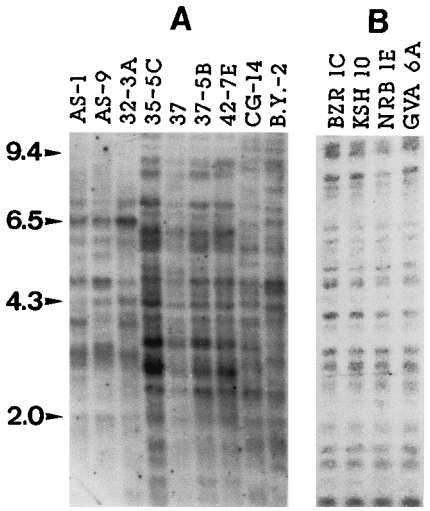


FIG. 5. Band patterns of genomic DNA of *C. gloeosporioides* isolates from avocado fruits (AS-1, AS-9, 32-3A, 35-5C, 37, 37-5B, 42-7E, CG-14, and B.Y.-2) (A) and almond fruits (BZR 1C; KSH 10, NRB 1E, and GVA 6A) (B) digested with *Pst*I and Southern blot hybridized with GcpR1 for nuclear-DNA analyses.

whereas three almond isolates (ALM-BZR-1C, -KSH-10, and -NRB-1E) had identical band patterns (Fig. 4). Among the avocado isolates of group 1, AVO-AS-1 and -AS-9 had nearly identical band patterns but differed from isolate -32-3A, which possessed an additional band of approximately 5.5 kb but lacked one of approximately 4.4 kb. Four other avocado isolates (AVO-34-4A, -47, -48-2B, and -CG-14) had a restriction fragment length polymorphism pattern different from those of the six isolates described above, whereas six additional almond isolates (ALM-GOZ-93, -GVA-6A, -GZT-1F, -KYZ-10A, -KN-17Q, and -NRB-1D) had patterns identical to those in Fig. 4 (data not shown).

**Comparison of different *C. gloeosporioides* isolates by nuclear-DNA analysis.** The patterns of nuclear hybridization of GcpR1 to *Pst*I-digested genomic DNA showed no polymorphisms among the representative isolates of almond (ALM-BZR-1C, -KSH-10, -NRB-1E, and -GVA-6A), whereas the avocado isolates were more variable (Fig. 5). The avocado isolates of group 1 (AVO-AS-1, -AS-9, and -32-3A) had similar nuclear-DNA banding patterns with minor differences in the regions of 4.0 to 6.0 kb (Fig. 5A). Isolates AVO-35-5C, -37, -37-5B, -42-7E, and -CG-14 had nearly identical banding patterns but differed from the other three isolates of group 1 and isolate AVO-B.Y.-2 (Fig. 5A). Isolate AVO-B.Y.-2 had banding patterns distinct from those of the other isolates according to nuclear-DNA analysis.

**Comparison of different *C. gloeosporioides* isolates by rDNA analysis.** Restriction digests of PCR-amplified rDNA from isolates of *C. acutatum*, *C. fragariae*, and *C. gloeosporioides* from strawberries, apples, avocados, and almonds were compared (Fig. 6). The restriction enzymes *Eco*RI and *Sau*3A distinguished between isolates of *Colletotrichum* spp., whereas the enzymes *Bam*HI, *Hae*III, *Hin*dIII, *Pst*I, and *Stu*I were unable to discriminate among species. *Eco*RI and *Sau*3A digest patterns of the amplified regions of all the *C. gloeosporioides* isolates from the different hosts, including the *C. fragariae* isolate, were identical, whereas only the *C. acutatum* rDNA was different from rDNA of the other isolates (Fig. 6).

**Comparison of almond and avocado *C. gloeosporioides* isolates by pathogenicity assay.** Detached almond and avocado fruits were spot inoculated with conidia from six avocado and five almond representative isolates. Artificially inoculated avocado fruit showed typical decay symptoms with avocado isolates, whereas the almond isolates caused only minor symptoms of decay manifested in darkening of inoculation zones. In almond fruits, almond isolates caused typical anthracnose lesions. Similar disease symptoms were observed on almond fruit inoculated with avocado isolates. A significant difference in sizes of lesions caused by avocado and almond isolates that were artificially inoculated on avocado fruit was observed (Table 4). The diameter (means ± standard error) of the lesions on avocado fruits that were inoculated with avocado isolates was 17.2 ± 3.6 mm, which was significantly different ($P = 0.018$, by using a $t$ test) from that of the lesions caused by almond isolates (2.6 ± 0.6 mm). Certain avocado isolates appeared more aggressive than others in causing faster decay development (Table 4). A significant difference in sizes of lesions caused by avocado and almond isolates that were artificially inoculated on almond fruit was also observed (Table 4). The diameter of the lesions on almond fruits that were inoculated with avocado isolates was 15.0 ± 1.3 mm, which was significantly different ($P = 0.056$) from that of the lesions caused by almond isolates (11.8 ± 1.4 mm).

## DISCUSSION

The main aim of this research was to determine by using molecular markers and a pathogenicity assay whether anthracnose of almond fruits and that of avocado fruits are caused by the same *C. gloeosporioides* population.

All of the Israeli *C. gloeosporioides* almond isolates, grouped together by nuclear genotyping with the repetitive DNA element GcpR1 from *C. lindemuthianum*, were identical according to ap-PCR and A+T-rich-DNA analyses. This suggests that a clonal population of the almond pathogen has probably spread throughout Israel without much variation. Uniformity of this population has been shown in previous studies on the basis of vegetative compatibility groupings and fungicide sensitivity assays of the almond isolates (17, 28). However, DNAs from eight U.S. *Colletotrichum* isolates that caused almond anthracnose were distinctly different from DNAs of the Israeli

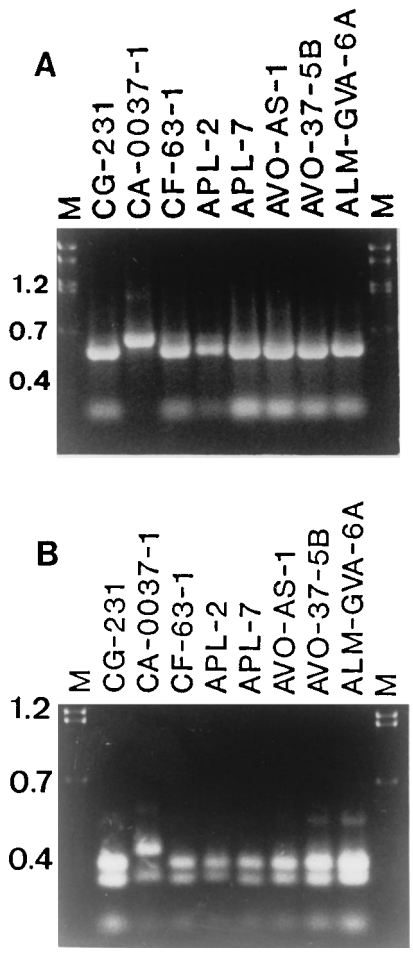


FIG. 6. Restriction digests obtained by using *Eco*RI (A) and *Sau*3A (B) with PCR-amplified rDNA from *C. gloeosporioides* (isolate CG-231) from strawberries, *C. acutatum* (isolate CA-0037-1) from strawberries, *C. fragariae* (isolate CF-63-1) from strawberries, *C. gloeosporioides* (isolates APL-2 and APL-7) from apples, *C. gloeosporioides* (isolates AS-1 and 37-5B) from avocado fruits, and *C. gloeosporioides* (isolate GVA-6A) from almond fruits. Lanes M contain DNA markers, with sizes (in kilobases) on the left.

TABLE 4. Lesion size on avocado and almond fruits artificially inoculated by isolates of *C. gloeosporioides* from avocado and almond[a]

| Origin | Isolate | Lesion diameter (mm) on fruit | |
|---|---|---|---|
| | | Avocado | Almond |
| Avocado | AVO-AS-9 | 9.5 ± 5.5 | 13.5 ± 1.5 |
| | AVO-AS-1 | 12.5 ± 2.5 | 14.0 ± 2.0 |
| | AVO-37 | 16.0 ± 4.0 | 16.0 ± 1.0 |
| | AVO-32-3A | 17.5 ± 2.5 | 17.0 ± 1.0 |
| | AVO-35-5C | 20.0 ± 4.5 | 13.5 ± 1.5 |
| | AVO-47 | 27.5 ± 2.5 | 16.0 ± 1.0 |
| Almond | ALM-GVA 6A | 2.2 ± 0.7 | 11.0 ± 1.0 |
| | ALM-BZR 1C | 2.4 ± 0.5 | 11.0 ± 1.0 |
| | ALM-GOZ 93 | 2.5 ± 0.3 | 13.5 ± 1.5 |
| | ALM-KSH 10 | 3.0 ± 0.8 | 10.0 ± 2.0 |
| | ALM-NRB 1E | 3.1 ± 0.5 | 13.5 ± 1.5 |

[a] Data are the means ± standard errors for two different experiments. In each experiment, 10 avocado fruits and 5 almond fruits were inoculated per isolate and incubated for 8 to 10 days at 20°C.

isolates. This indicates that the U.S. population of isolates that cause almond anthracnose, not yet defined taxonomically, may comprise species or genotypes of *Colletotrichum* other than those of the Israeli *C. gloeosporioides* population.

The U.S. and Israeli isolates of *C. gloeosporioides* from avocados were more diverse than the almond isolates, indicating that there are numerous subpopulations. Multiple phenotypes were observed on the basis of results obtained from three different molecular approaches. However, no similarities were found between any avocado isolate and the Israeli almond isolates by using ap-PCR, nuclear-DNA, and A+T-rich-DNA analyses. This result is a further indication that the almond population is distinct.

In previous studies with populations of *C. acutatum* and *C. fragariae* from strawberries, little genomic variation within each species was observed by using different molecular methods (10, 11). In contrast, a high level of diversity was observed in populations of *C. gloeosporioides* from apples, avocados, and strawberries and *Colletotrichum musae* from bananas (5, 10, 11, 13, 23). Therefore, the uniformity of Israeli almond isolates may be due to an asexually reproducing population. Multiple genotypes in sexually reproducing *C. gloeosporioides* (*Glomerella cingulata*) populations may be derived from genetic recombination; however, asexually reproducing *Fusarium oxysporum* f. sp. *lycopersici* has been reported to possess multiple genotypes (7).

A fourth molecular approach based on limited restriction digest analyses of PCR-amplified rDNA did not distinguish between the *C. gloeosporioides* isolates from a diverse host range including almonds, apples, avocados, and strawberries. An additional representative isolate of *C. fragariae* from strawberries had the same restriction pattern as the *C. gloeosporioides* isolates but differed from an isolate of *C. acutatum* that was also from strawberries. Previously it was shown that, on the basis of sequence analyses of conserved regions of rDNA (29), *C. fragariae* may fit within the group species of *C. gloeosporioides*. Additional sequence data for amplified rDNA from the different populations of *C. gloeosporioides* and other *Colletotrichum* species may be necessary for further comparisons.

Disease symptoms developed in almond and avocado fruits after artificial inoculations with isolates of *C. gloeosporioides* from both hosts. Despite this fact, it is not certain whether cross-infections of both fruits by each pathogen occur naturally in the field. Recently it was shown that isolates of *C. gloeosporioides* from a wide range of tropical fruits, including avocado, durian, guava, mango, mangosteen, pini jambu, and rambutan, had cross-infection potential (1). However, artificial inoculations performed in that study were done on detached young leaves similarly to our artificial inoculations done on detached fruit. This may explain the potential for cross-infection of almonds by avocado isolates under artificial conditions. It should be noted, however, that according to the molecular analyses done in this study, all cultures isolated from naturally infected almonds in Israel were uniform. This indicates that the almond pathogen in Israel is specific for the almond host under field conditions.

Certain avocado isolates infected avocado fruit at different rates, which demonstrates pathogenic diversity coupled with genotypic diversity of the population. However, no phenotypic correlations between the subgroups within the avocado isolates could be made. Although the avocado isolates used in this study were identified as *C. gloeosporioides* by morphotaxonomic criteria, other species such as *C. acutatum* may be involved in pathogenesis, as has been shown in New Zealand (15).

PCR amplification of total genomic DNA and nuclear DNA and partial mtDNA analyses for comparing almond and avocado isolates of *C. gloeosporioides* complemented each other with similar results, confirming the heterogeneity of avocado isolates from two different geographic regions worldwide and the uniformity of the Israeli almond isolates. Thus, ap-PCR, A+T-rich-DNA analyses, and the GcpR1 repetitive element, which have been used for systematics and taxonomy in the genus *Colletotrichum* (10) and as potential diagnostic tools for differentiating causal agents of strawberry anthracnose disease (10, 11), may also be valuable for determining the genetic diversity of other *Colletotrichum* populations.

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