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Potential of Cross-infection of *Colletotrichum* Species Causing Anthracnose in Persimmon and Pepper

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Ninety isolates of *Colletotrichum* species from new persimmon tree twigs and 50 isolates from pepper plant fruits were isolated via single-spore isolation. Of the 140 isolates, 26 were examined for mycelial growth, carbendazim sensitivity, and ITS sequence. Four of the isolates from the persimmon trees, which were cultivated exclusively in an orchard, showed fast mycelial growth and sensitivity to carbendazim, while five of the pepper isolates showed slower mycelial growth and were resistant to the fungicide. However, 17 isolates from persimmon trees cultivated with pepper plants in the same orchard showed slow mycelial growth like the pepper isolates and they were sensitive to carbendazim like the persimmon isolates. ITS sequence analysis of these 27 isolates led to the identification of the 22 persimmon isolates as *C. gloeosporioides* and the five pepper isolates as *C. acutatum*. PCR with species-specific primers confirmed that the 90 isolates from persimmon were *C. gloeosporioides* whereas the 50 isolates from pepper were *C. acutatum*. The 90 persimmon isolates of *C. gloeosporioides* and 50 pepper isolates of *C. acutatum* were compared by a wound inoculation test to determine their capacity for host cross-infection. All of the *C. acutatum* isolates from pepper caused typical symptoms of anthracnose on the fruits of pepper plants and twigs of persimmon; they differed from the *C. gloeosporioides* isolates from persimmon, more than 90% of which were able to infect only persimmon. Amplified fragment length polymorphism analysis revealed the existence of two groups (*C. gloeosporioides* and *C. acutatum* isolates group). At 80% genetic similarity, the *C. gloeosporioides* group was defined within four clusters, while the *C. acutatum* group was within three clusters. However, these clusterings were unrelated with the virulence of *Colletotrichum* species against pepper fruits.

Keywords : *Colletotrichum acutatum*, *C. gloeosporioides*, cross-infection, pepper, persimmon

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Morphological characteristics such as colony morphology, conidial shape, the presence or absence of setae and sclerotia, and the appressorium shape and size have been used to differentiate species in the genus *Colletotrichum*. Morphological traits, however, are highly variable among isolates and often subject to interpretation. Other characters, such as growth rate and sensitivity to benomyl, have been useful in differentiating between *C. acutatum* and *C. gloeosporioides*. *Colletotrichum gloeosporioides* is a ubiquitous, proliferate, and economically important pathogen that causes substantial yield losses due to fruit decay and vegetative tissue damage in a variety of plant species. Anthracnose, dieback, root rot, leaf spot, blossom rot, and seedling rot caused by *C. gloeosporioides* have been reported for a wide range of plants including avocado, almond, peach (Freeman et al., 1998), pepper (Park and Kim, 1992; Manadhar et al., 1995), citrus (Timmer et al., 1994), rubber tree (Brown and Soepena, 1994), and strawberry (Denoyes and Baudry, 1995). In comparison, *C. acutatum* affects many of the hosts that are susceptible to other *Colletotrichum* species (spp.). For example, strawberry plants may be infected by *C. acutatum*, *C. gloeosporioides*, or *C. fragariae* (Freeman and Katan, 1997; Gunnell and Gubler, 1992). Among citrus plants, whereas *C. gloeosporioides* is a common saprophyte and may cause postharvest anthracnose from preharvest quiescent infections, *C. acutatum* causes distinct diseases in different species, such as postbloom fruit drop and key lime anthracnose (Timmer et al., 1994). Interestingly, in *Colletotrichum*, multiple hosts can be affected by a single species, and multiple species can affect a single host.

A number of cross-infection studies have been conducted using *C. gloeosporioides* and *C. acutatum*, and a wide range of hosts (Mass and Howard, 1985; Alahakoon et al., 1994; Freeman et al., 1996). The various virulence of *C. gloeosporioides* isolated from several hosts was shown in other hosts (Quimio and Quimio, 1975; Freeman and Shabi, 1996). Quimio and Quimio (1975) found differences in the extent of virulence among 11 isolates of *C. gloeosporioides* on green carabao mango. They also reported that isolates of *C. gloeosporioides* from mango, citrus, and

papaya caused infections on the fruits of each of these hosts; however, there was variability in the degree of virulence. In fact, some isolates of *C. gloeosporioides* from mango did not infect banana or guava. Fagan (1979) was unable to cross-infect citrus flowers with an inoculum from senescent grapefruit leaves. Isolates of *C. gloeosporioides* from almond, apple, mango, and avocado inoculated into detached apple, avocado, almond, mango, and nectarine were shown to produce successful cross-infections (Freeman and Shabi, 1996). Furthermore, using conidial suspensions of isolates of *C. acutatum* from apple and peach, Freeman and Shabi (1996) inoculated detached nectarine, mango, avocado, almond, and apple fruits. All of the isolates produced lesions on the fruits, but it was necessary to wound the mango fruit to produce an infection. Peres et al. (2002) found that a *C. acutatum* isolate from strawberry produced lesions on wounded, detached avocado, guava, papaya, mango, and some fruits but not on banana. Isolates of *C. acutatum* from strawberry also produced lesions on wounded and unwounded fruits from pear, peach, nectarine, and apple (Freeman et al., 2001). Thus, it appears that *Colletotrichum* spp. have a broad host range and are relatively nonspecific. As many hosts susceptible to *Colletotrichum* spp. are cultivated worldwide, the losses in fields where mango, avocado, coffee, papaya, and citrus are grown in close proximity could be enormous (Freeman et al., 1998).

In Sangju, Korea, there are several orchards where persimmon trees have been cultivated with pepper. Severe infections caused by anthracnose pathogens have been observed in both pepper and persimmon. In particular, persimmon anthracnose has had disastrous effects on the cultivation and production of persimmon (Lee et al., 2001). Kwon et al. (2000) reported the cultural characteristics of *C. gloeosporioides*, which causes persimmon anthracnose. In pepper anthracnose, the pathogen was identified as *C. acutatum* based on such characteristics as morphology, mycelial growth, benomyl sensitivity, and an analysis of the ITS region using molecular markers (Kim et al., 2008). Because crops such as pepper are often cultivated in close proximity to persimmon orchards in Sangju, Korea, it is important to determine whether these hosts can serve as inoculum sources for one another. In this study, we identified isolates obtained from persimmon based on their mycelial growth characteristics and carbendazim sensitivity, as well as a phylogenetic analysis of their ITS regions by PCR using species-specific primers. We also examined the virulence of each isolate on their respective hosts and their potential for cross-infection.

Materials and Methods

Isolation of the anthracnose pathogens. To obtain isolates through single-spore isolation, pieces of diseased persimmon twigs and fruits from pepper plants grown in persimmon orchards were surface-sterilized with 2% sodium hypochlorite for 1 min then washed twice with sterile distilled water (SDW) and placed in plastic containers (30×20×10 cm, W×L×H) lined with three paper towels soaked with 100 ml of distilled water to maintain high humidity (>95%, RH). After incubation, the spores produced on the pieces were harvested with SDW and washed with SDW twice. The spore concentration was adjusted to 1×10^6 conidia/ml and 50 μl of the resulting suspension was spread on potato dextrose agar (PDA; Difco, Franklin Lakes, NJ, USA) supplemented with 300 μg/ml streptomycin. After 3 days of incubation at 25°C, mycelial discs at the margins of small colonies were removed and subcultured. The isolates were maintained on PDA slants at 4°C until use.

Mycelial growth and carbendazim sensitivity. To measure mycelial growth, a 5-mm-diameter mycelial disc taken from the margin of a 7-day-old culture grown on PDA was inoculated in the center of PDA plates. The colony diameter of each isolate was measured daily for 1 week at 25°C. To determine the carbendazim sensitivity, agar blocks were taken from the margins of the colonies grown on PDA at 25°C for 7 days and transferred to PDA plates containing the fungicide at 10 μg/ml. Carbendazim (a.i. 95%) was dissolved in dimethyl sulfoxide (DMSO) and added to the medium at the indicated concentration just before pouring into the Petri plates. DMSO alone was used as a control. The final concentration of DMSO was <1% in all cases. After incubation at 25°C for 7 days, the colony diameter in each culture was evaluated on PDA with or without the fungicide. Three replicates were prepared for each isolate in both experiments.

Cross-infection test. Detached persimmon twigs and detached fruits from pepper plants were used to assess the pathogenicity of each isolate. The twigs and fruits were wounded with a pin and inoculated with a 5-μl drop of a conidial suspension adjusted to 5×10^5 conidia/ml. Conidia were harvested from cultures of two species of *Colletotrichum* incubated at 25°C for 10 days. The inoculated materials were placed in plastic containers (30×20×10 cm, W×L×H) lined with three paper towels soaked with 100 ml of SDW to maintain high humidity. Lesion length was measured after 10 days at 25°C.

Genomic DNA Extraction. Total DNA was extracted from mycelia obtained from the PDA cultures after 10 days of growth at 25°C. Aerial mycelia were harvested from the culture plates using a sterile transfer needle and placed in

sterile 1.5-ml microcentrifuge tubes containing 200 μl of extraction buffer (0.2 M Tris-HCl, 0.25 M NaCl, 25 mM EDTA, and 2% SDS, pH 8.5). The tubes were placed in a boiling water bath for 5 min and then cooled to 25°C. Then, 200 μl of phenol that was equilibrated with extraction buffer (v/v) and 200 μl of chloroform were added. The tubes were subsequently vortexed for 4 min and centrifuged at 13,000× *g* for 5 min. The supernatants were then transferred to fresh sterile 1.5-ml tubes, and 200 μl of chloroform was added; each mixture was then vortexed for 30 s and centrifuged at 13,000× *g* for 15 min. The supernatants were then extracted with 200 μl of isopropanol and centrifuged at 13,000× *g* for 15 min. Next, the nucleic acid pellets were washed with 70% ethanol, air-dried for 15 min, and resuspended in 50 μl of TE buffer (10 mM Tris-HCl and 0.1 mM EDTA, pH 8.5).

Sequencing of the ITS region. The primers ITS5 and ITS4-3 were used for amplification of the ITS region (Kang et al., 2005). Amplification was conducted in a total reaction volume of 25 μl using a PCR kit (Bioneer Inc., Daejeon, Korea). The parameters used were as follows: a 2-min hold at 95°C followed by 40 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C with a final extension for 5 min at 72°C. The amplicons were column-purified using a PCR purification kit (Atman Biosciences, Uiwang, Korea) and cycle-sequenced by Eugentech Inc. (Taejon, Korea). The sequences were analyzed using both EditSeq and MegAlign (DNASTAR Inc., Madison, WI, USA).

PCR with species-specific primers for the identification of *Colletotrichum* spp. The primers CgInt (5'-GGC CTC CCG CCT CCG GGC GG-3') and Ca1-3 (5'-CAG GGG AAG CCT CTC GCG GGC CT-3') were designed based on sequence similarities in the ITS1 region between *C. gloeosporioides* and *C. acutatum* (Mills et al., 1992; Kim et al., 2008). CgInt (specific for *C. gloeosporioides*) or Ca1-3 (specific for *C. acutatum*) was used with the backward primer ITS4-3, which is specific for both *C. gloeosporioides* and *C. acutatum*. Each reaction mix (30 μl) contained 50 ng of DNA, 1 M each primer, and 15 ml of PCR Master Mix (Promega, Madison, WI, USA). Amplification was conducted with at least three replicates as follows: one cycle of 4 min at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at 65°C, and 1 min at 72°C with a final 7-min extension at 72°C. The products were then separated on 0.7% agarose gels.

Amplified fragment length polymorphism (AFLP) analysis. AFLP analysis was performed as described with modifications (Vos et al., 1995). About 1 μg of genomic DNA was digested with *Eco*RI and *Mse*I (New England Biolabs UK Ltd., Hertfordshire, UK). The fragments were then ligated to double-stranded restriction site-specific adaptors using T4 DNA ligase (Takara, Shiga, Japan) and diluted 1:10 with TE buffer. Pre-selective PCR was performed using E00 (5'-GAC TGC GTA CCA ATT C-3') and M02 (5'-GAT GAG TCC TGA GTA AC-3') in a reaction mix (25 μl) containing 0.5 μM each primer, 1X *Taq* buffer (2 mM Tris-HCl, 10 mM KCl, 10 μM EDTA, 0.1 mM DTT, 0.05% NP-40, 0.05% Tween 20, 5% glycerol, and 2 mM $MgCl_2$), 0.2 mM dNTPs, 1 U of *Taq* polymerase (Enzynomics, Daejeon, Korea), and 2 μl of diluted DNA. PCR was performed as follows: 5 min at 95°C followed by 20 cycles of 30 s at 95°C, 1 min at 56°C, and 1 min at 72°C with a final 7-min extension at 72°. For selective PCR, 2 μl of a 1:10 dilution of each pre-selective product was amplified using E42 (5'-GAC TGC GTA CCA ATT CTC-3') and M213 (5'-GAT GAG TCC TGA GTA ACA G-3') in a 25-μl volume with the following parameters: pre-denaturation at 95°C for 5 min followed by 12 cycles of denaturation at 95°C for 30 s, a 0.7°C step-wise reduction in the annealing temperature from 65°C for 30 s, and extension at 72°C for 1 min, plus an additional 23 cycles with annealing at 56°C for 30 s. The AFLP fragments were size-fractionated in 4% polyacrylamide sequencing gels and visualized using the Silverstar® Staining System (Bioneer Inc.). The AFLP profiles were analyzed using GelCompar II (Applied Maths BVBA, Sint-Martens-Latem, Belgium). A phylogenetic tree was constructed using Pearson's correlation coefficient and cluster analysis by the unweighted pair group method with arithmetic averages (UPGMA).

Results

Characteristics of the persimmon and pepper isolates. Mycelial growth, carbendazim sensitivity, and virulence were investigated using isolates obtained from persimmon and pepper. The persimmon isolates were divided into two groups: the group 1 isolates including 5 isolates from PER1 to PER5 in Table 1 were taken from diseased twigs in an orchard where only persimmon trees were cultivated, while the group 2 isolates including 17 isolates from PPF1 to PPF17 were taken from an orchard in which persimmon trees and pepper plants were cultivated simultaneously. As shown in Table 1, none of the isolates obtained from persimmon showed virulence in the pepper fruits or resistance to carbendazim. However, the isolates obtained from diseased pepper fruits grown in a persimmon orchard showed high virulence on pepper fruits and were able to grow on PDA containing 10 μg/ml carbendazim. In terms of the mycelial growth of the persimmon isolates, a difference was detected between the group 1 and group 2 isolates. All of the group 2 isolates grew less than 50.0 mm during 7 days of incubation

Table 1. Virulence on pepper fruits, mycelial growth on PDA, and carbendazim sensitivity of *Colletotrichum* isolates from persimmon and pepper, respectively

Isolate[a]	Host Plant	Virulence[b] on Pepper Fruit (mm)	Mycelial Growth[c] (mm)	Carbendazim Sensitivity[d] (mm)
PER 1	Persimmon	0.0	65.6	0.0
PER 2	Persimmon	0.0	64.3	0.0
PER 3	Persimmon	0.0	61.2	0.0
PER 4	Persimmon	0.0	62.6	0.0
PER 5	Persimmon	0.0	64.5	0.0
PPF 1	Persimmon	0.0	40.9	0.0
PPF 2	Persimmon	0.0	44.9	0.0
PPF 3	Persimmon	0.0	45.4	0.0
PPF 4	Persimmon	0.0	43.3	0.0
PPF 5	Persimmon	0.0	41.1	0.0
PPF 6	Persimmon	0.0	42.3	0.0
PPF 7	Persimmon	0.0	47.3	0.0
PPF 8	Persimmon	0.0	43.4	0.0
PPF 9	Persimmon	0.0	39.5	0.0
PPF 10	Persimmon	0.0	43.6	0.0
PPF 11	Persimmon	0.0	48.7	0.0
PPF 12	Persimmon	0.0	37.4	0.0
PPF 13	Persimmon	0.0	42.8	0.0
PPF 14	Persimmon	0.0	39.7	0.0
PPF 15	Persimmon	0.0	38.4	0.0
PPF 16	Persimmon	0.0	50.6	0.0
PPF 17	Persimmon	0.0	43.7	0.0
PEP 1	Pepper	15.9	37.5	18.3
PEP 2	Pepper	15.7	40.1	14.5
PEP 3	Pepper	27.6	42.7	15.1
PEP 4	Pepper	16.8	41.5	16.2

[a] Isolates PER 1 to PER 5 were obtained from persimmon trees grown in orchards without pepper, while isolates PPF 1 to PPF 17 were from persimmon trees grown in orchards where persimmon and pepper were cultivated simultaneously. Isolates PEP 1 to PEP 4 were obtained from pepper plants grown in persimmon orchards.

[b] Pepper fruits were inoculated with a conidial suspension (5×10^5 conidia/ml) by dropping 5 μl of the suspension onto sites of wounding. The lesion lengths were measured after 10 days of incubation at high humidity in a plastic box at 25°C.

[c] The colony diameter of the *Colletotrichum* species on PDA was investigated after incubation for 7 days at 25°C.

[d] After incubation at 25°C for 7 days, the colony diameter in each culture was evaluated on PDA containing 10 μg/ml carbendazim.

on PDA at 25°C, except for isolate PPF 16, which showed the same mycelial growth tendency as the pepper isolates. In comparison, the group 1 isolates grew faster than the pepper and group 2 isolates, with a mean growth of 63.6 mm.

ITS sequence analysis and species-specific PCR. The phylogenetic relationships among the *Colletotrichum* isolates were inferred by UPGMA analysis of the nucleotide sequences of the amplified ITS regions (Fig. 1). According to our results, the *Colletotrichum* isolates fit well into two distinct groups: *C. acutatum* and *C. gloeosporioides*. While all of the pepper isolates possessed the ITS sequence of *C. acutatum*, all of the persimmon isolates belonged to the *C. gloeosporioides* group. A portion of the ITS region was amplified from the pepper and persimmon *Colletotrichum* isolates using primers designed for *C. acutatum* (Ca1-1) or *C. gloeosporioides* (CgInt). The primer sequences were derived from regions showing species-specific variation in the ITS region. Using the *C. acutatum*-specific primer Ca1-1 and the reverse primer ITS4-3, a 499-bp fragment was amplified from each of the pepper isolates PEP 1, PEP 2, and PEP 3 in Fig. 2. Using CgInt and ITS4-3, a 466-bp fragment was amplified from each of the persimmon isolates, including the 16 persimmon isolates in group 2 (PPF 1-PPF 16) and the five persimmon isolates in group 1 (PER 1-PER 5). Furthermore, it was confirmed that the others of the 90 persimmon isolates were *C. gloeosporioides* while the others of 50 pepper isolates were *C. acutatum*.

Comparison of the cross-infection potential. The *Colletotrichum* spp. analyzed in this study corresponded to 140 isolates, 50 of which were *C. acutatum* isolates from pepper and 90 of which were *C. gloeosporioides* isolates from persimmon. Of the 90 isolates from persimmon, 45/46 in group 1 and 43/44 in group 2 produced typical symptoms of anthracnose on detached persimmon twigs (Table 2). In contrast, among the pepper fruits, only four of the *C. gloeosporioides* isolates from group 1 and six *C. gloeosporioides* isolates from group 2 showed symptoms of anthracnose with a mean lesion size of 2.6 mm on pepper fruits. Nonetheless, out of the 50 pepper isolates identified as *C. acutatum*, 48 showed strong virulence, producing typical symptoms of anthracnose on pepper fruits. Moreover, all of the isolates produced symptoms of anthracnose on new persimmon twigs, with reduced virulence compared to the persimmon isolates. The mean lesion length caused by the pepper isolates was 9.0 mm on new persimmon twigs, compared to 13.0 and 12.4 mm for the persimmon isolates in groups 1 and 2, respectively.

AFLP analysis. Using AFLP, 140 isolates of *Colletotrichum* spp. from persimmon and pepper were analyzed for genetic diversity, and a dendrogram was constructed by the UPGMA method (Fig. 3). At 80% genetic similarity, seven clusters were defined and gathered into two groups: the *C. acutatum* isolates and the *C. gloeosporioides* isolates. Minimum genetic similarity occurred between the two groups at 0.009. The *C. acutatum* isolate group was divided into three clusters. Cluster B contained only *C. acutatum* PEP 14, which showed the greatest virulence on twigs of persim-

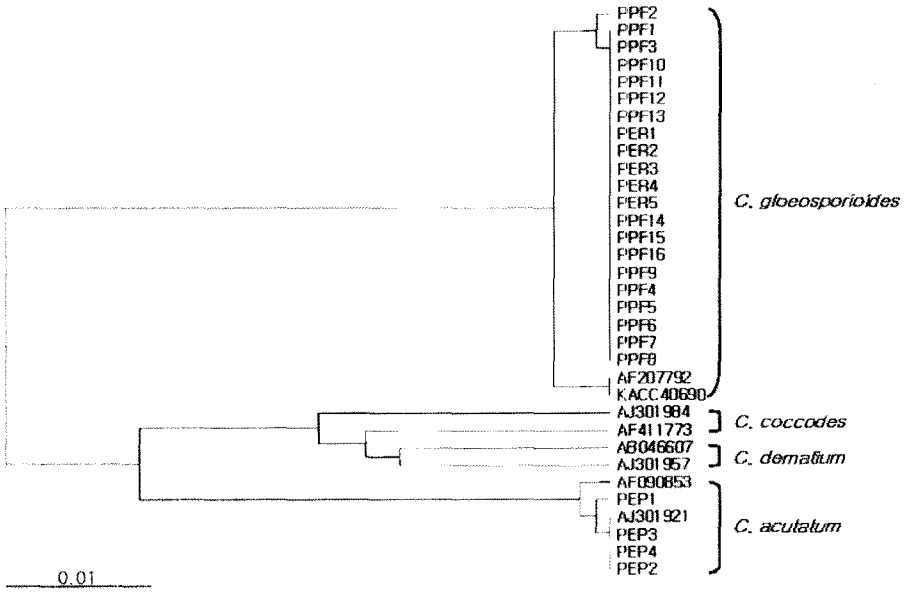

Fig. 1. An ITS-based phylogenetic tree of the *Colletotrichum* isolates. The tree was constructed using cluster analysis with the unweighted pair group method with arithmetic averages (UPGMA). The branch orders were similar in all tree-construction approaches used. The scale bar indicates an estimated 1% sequence divergence.

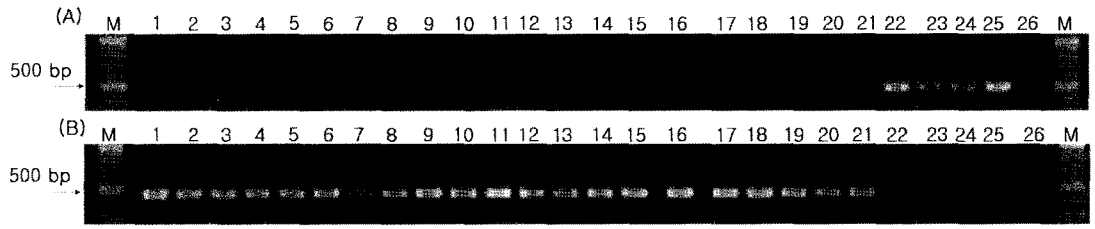

Fig. 2. Amplification of a species-specific fragment from fungal DNA using the primers Ca1-3 and ITS4-3 for *Colletotrichum acutatum* (A) or CgInt and ITS4-3 for *C. gloeosporioides* (B). Lane: M, size marker; 1, PER 1; 2, PER 2; 3, PER 3; 4, PER 4; 5, PER 5; 6, PPF 1; 7, PPF 2; 8, PPF 3; 9, PPF 4; 10, PPF 5; 11, PPF 6; 12, PPF 7; 13, PPF 8; 14, PPF 9; 15, PPF 10; 16, PPF 11; 17, PPF 12; 18, PPF 13; 19, PPF 14; 20, PPF 15; 21, PPF 16; 22, PEP 1; 23, PEP 2; 24, PEP 3; 25, PEP 4; 26, *C. gloeosporioides* KACC40690.

Table 2. Isolates of *Colletotrichum* species summarized according to host plant and their virulence on persimmon and pepper

Host Used in this Study[a]	Species, Collection Host	Number of Isolates		
		Pathogenic[e]	Nonpathogenic	Total
Pepper	*C. gloeosporioides*, persimmon only[b]	4	42	45
	C. gloeosporioides, persimmon with pepper[c]	6	38	44
	C. acutatum, pepper with persimmon[d]	48	2	50
Persimmon	*C. gloeosporioides*, persimmon only	45	1	46
	C. gloeosporioides, persimmon with pepper	43	1	44
	C. acutatum, pepper with persimmon	50	0	50

[a] Persimmon and pepper were used as the host plants for the virulence test of all *Colletotrichum* isolates.
[b] Obtained from diseased persimmon twigs in an orchard where persimmon was cultivated alone.
[c] Obtained from diseased persimmon twigs in an orchard where persimmon was cultivated with pepper plants.
[d] Obtained from diseased pepper fruits in an orchard where persimmon was cultivated with pepper plants.
[e] Persimmon twigs and pepper fruits were wounded by a pin and inoculated by dropping 5 μl of a conidial suspension adjusted to 5×10^5 conidia/ml onto the wound. Lesion length was measured after incubation of the inoculated materials at 25°C for 10 days in plastic containers (30×20×10 cm, W×L×H) lined with three paper towels soaked with 100 ml of distilled water to maintain high humidity.

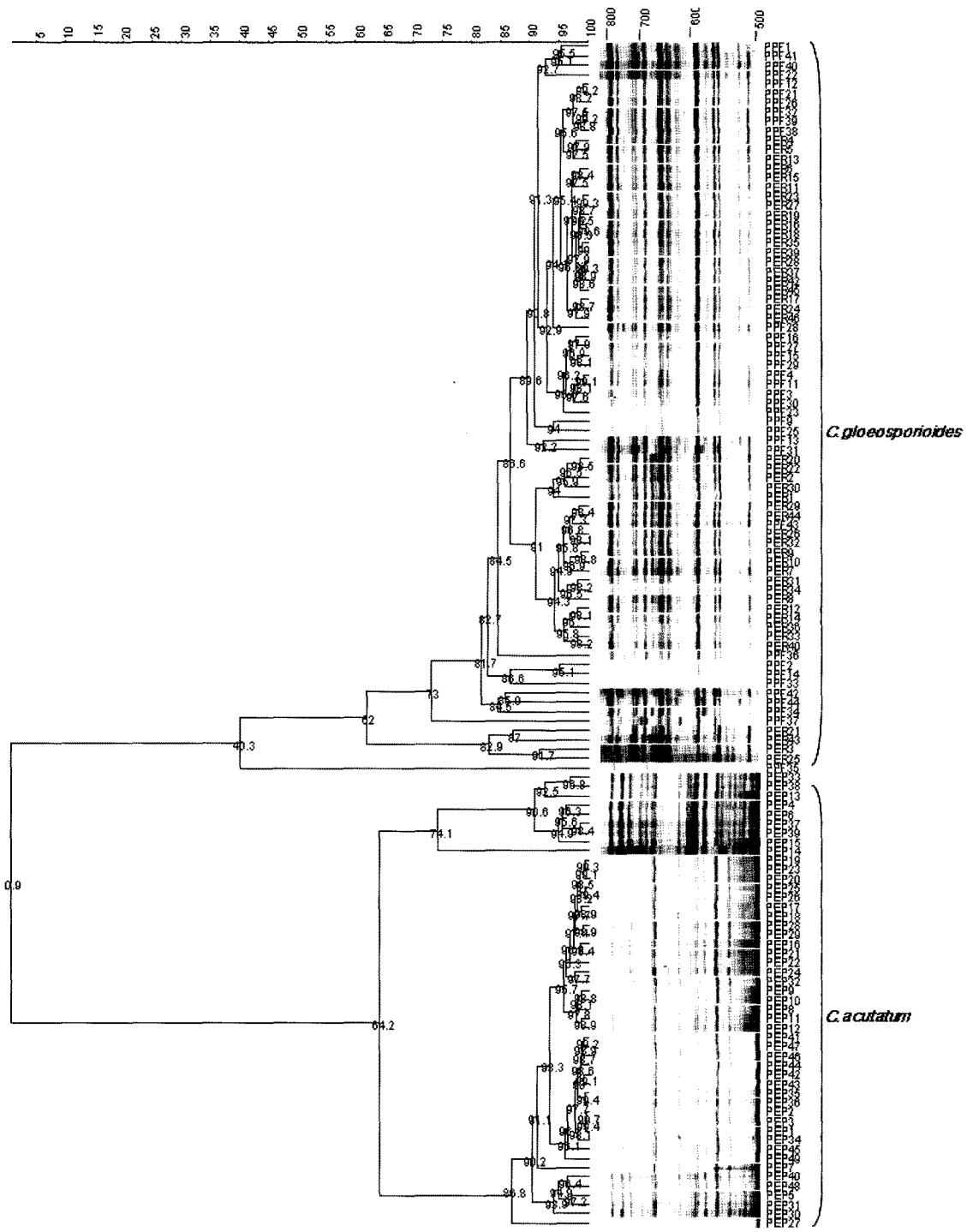

Fig. 3. Consensus dendrogram generated by 1,000 bootstrap reiterations using GelCompar II (Applied Maths BVBA) to analyze the AFLP data for 140 *Colletotrichum* species isolates. The dendrogram was constructed using Pearson's correlation coefficient and cluster analysis with UPGMA.

mon among the 50 *C. acutatum* isolates. Clusters A and C contained 41 and 8 isolates of *C. acutatum*, respectively. The *C. gloeosporioides* isolate group was divided into four clusters. Clusters D and F each contained a single isolate: *C. gloeosporioides* PPF 35 and PPF 37, respectively. Four isolates, *C. gloeosporioides* PER 3, 21, 25, and 43, were included in cluster E. Cluster G, which included 84 isolates of *C. gloeosporioides*, was subdivided into nine clusters at 90% genetic similarity.

Discussion

The *Colletotrichum* spp. identified in this study were divided into two groups: *C. gloeosporioides* and *C. acutatum*. All of the *C. gloeosporioides* isolates were obtained from persimmon, while those of *C. acutatum* were obtained from pepper. The *Colletotrichum* spp. were separated according to their host plant preferences. Although we were unable to detect cross-infection by the *Colletotrichum* spp. between persimmon and pepper in the field, it was inferred that the *Colletotrichum* spp. could attack either host plant due to low host specificity. In reality, our cross-infection results indicated some variation in the host preference of the *C. gloeosporioides* and *C. acutatum* isolates from persimmon and pepper. Whereas the *C. gloeosporioides* isolates were much more pathogenic on persimmon than on pepper, the *C. acutatum* isolates were able to infect both hosts. In addition, there was a clear distinction between the *C. gloeosporioides* and *C. acutatum* isolates from persimmon and pepper based on the sequences of their ITS regions and AFLP data. However, isolates of *C. gloeosporioides* were not divided into different two groups in AFLP data, as one was from the orchard where only persimmon trees were cultivated and the other was from the orchard with both of crops such as persimmon and pepper.

As shown in Table 2, while 88 isolates of *C. gloeosporioides* were able to produce symptoms on their original host, but only 10 isolates were able to do on pepper. Several previous reports have also noted the variation in virulence of *C. gloeosporioides* isolates (Darvas et al., 1987; Freeman and Shabi, 1996). These differences may be ascribed to adaptations of the pathogen to its host, allowing it to become more virulent and to overcome the host's defense mechanisms (Alahakoon et al., 1994). These adaptations may be highly host-specific, such that the ability of the pathogen to infect other hosts is eliminated. In contrast, the *C. acutatum* isolates used in this study appeared to be pathogenic toward both persimmon and pepper. It was previously reported that the causative pathogen of pepper anthracnose in Korea was *C. acutatum* (Kim et al., 2008); moreover, *C. acutatum* has been shown to have a broad host range and be relatively nonspecific (Peres et al., 2005). In research conducted in Israel, the inoculation of isolates of *C. acutatum* from anemone and strawberry into each host resulted in typical symptoms of anthracnose on both host plants (Freeman et al., 2000). In contrast, although isolates of *C. acutatum* were recovered from pepper, eggplant, tomato, bean, and weeds of the genera *Vicia* and *Conyza*, disease symptoms occurred only in strawberry (Freeman et al., 2001). Thus, *C. acutatum* was reported to parasitize and cause disease on other hosts, or, alternatively, to survive on other crops or on weeds without producing symptoms. Than et al. (2008) presented the cross-infection potential of *C. acutatum* isolates from strawberry to fruits of the pepper cv. Bangchang, which are susceptible to anthracnose caused by *C. acutatum*.

In the orchards of Sangju, Korea, most farmers cultivate pepper plants between their persimmon trees. Based on our results, although *C. gloeosporioides* from persimmon was not able to infect pepper, *C. acutatum* isolated from pepper could infect not only pepper but also persimmon. This suggests that in the persimmon orchards of Sangju, *C. acutatum* may cross-infect both pepper and persimmon plants.

Farmers could use benzimidazole fungicides to control persimmon anthracnose. Use of these fungicides might play a role in selection in persimmon orchards. Benzimidazole fungicides are largely effective against anthracnose, except in pepper (Lim and Choi, 2006; Kim et al., 2007). Even in our *in vitro* experiments, all of the *C. gloeosporioides* isolates appeared to be sensitive to carbendazim at 10 μg/ml, although the *C. acutatum* isolates were resistant to the fungicide as reported by Kim et al. (2007). Mycelial growth and conidia production are routinely used to assess the field fitness of plant pathogens (Sacristán and García-Arenal, 2008). Although the mycelial growth of *C. gloeosporioides* was reported to be faster than that of *C. acutatum*, conidia production was lower in *C. gloeosporioides* than in *C. acutatum* (Agostini et al., 1992; Berstein et al., 1995; Liyanage et al., 1992; Kim et al., 2008). Thus, it was suggested that *C. acutatum* be considered as a major pathogen in persimmon orchards, where two species of *Colletotrichum*, namely *C. gloeosporioides* and *C. acutatum*, are distributed, due to its strong resistance to carbendazim and conidia production.

Our cross-infection analysis was conducted in the laboratory using detached and wounded twigs and fruits. Despite the significant potential for cross-infection on the detached materials used in this study, it remains to be determined whether isolates from a different host pose a serious threat in the field, since our inoculation studies were carried out under optimum conditions for infection by the pathogen. There is currently no proof that the cross-infection of different hosts by *C. acutatum* actually occurs under field conditions; thus, additional studies are required.

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