Plant Pathol. J. 23(3) : 187-192 (2007)

The Plant Pathology Journal

Analyses of the Less Benzimidazole-sensitivity of the Isolates of *Colletotrichum* spp. Causing the Anthracnose in Pepper and Strawberry

Yun-Sik Kim[1], Ji Young Min[1], Beum Kwan Kang[1,4], Ngyeun Van Bach[1,5], Woo Bong Choi[2], Eun Woo Park[3] and Heung Tae Kim[1]*

[1]*Department of Plant Medicine, Chungbuk National University, Cheongju 361-763, Korea*
[2]*Department of Biotechnology and Bioengineering/Biomaterial Control, Dong-Eui University, Busan 614-714, Korea*
[3]*Department of Applied Biology and Chemistry, Seoul National University, Seoul 151-921, Korea*
[4]*Bayer CropScience Korea Ltd., 532-6 Dongcheon-ri, Jinwi-myeon, Pyeongtak 451-864, Korea*
[5]*Saigon Pesticide Co., RQ1. Tan Thuan Dong Ward, District 7, Ho Chi Minh, Vietnam*

(Received on July 16, 2007; Accepted on August 10, 2007)

The anthracnose disease on pepper fruits in Korea was caused by *Colletotrichum acutatum* as well as *C. gloeosporioides*. Since *C. acutatum* showed less sensitivity to benomyl, it was analyzed whether the less sensitivity was given by the same mechanism for the fungicide resistance of *C. gloeosporioides*. The isolates of *C. acutatum* were less sensitive to the three benzimidazole fungicides tested, benomyl, carbendazim, and thiophanate-methyl. However, the of *C. acutatum* isolates were different from the resistant isolates of *C. gloeosporioides* in their response to diethofencarb, one of *N*-phenylcarbamates; the former was still less sensitive to diethofencarb than the latter. The differences in the resistance mechanisms in two species were conspicuous in sequence analysis of the *tub2* genes. The genes from *C. acutatum* did not show any non-synonymous base substitutions at the regions known to be correlated with the benzimidazole-resistance. All of these data may indicate that the less sensitivity of *C. acutatum* to benomyl is based on different mechanism(s) from that of *C. gloeosporioides*.

Keywords : benzimidazole resistance, *Colletotrichum acutatum*, pepper anthracnose, *tub2* sequence

The pepper anthracnose is the fungal disease resulting in one of the most serious damages to the pepper production in Korea. Annual losses resulted from this disease are estimated to be more than 100 million US$, one tenth of total pepper production (Kim and Park, 1988). Several species of *Colletotrichum*, *C. acutatum*, *C. coccodes*, *C. dematium*, and *C. gloeosporioides*, were known to have the ability to infect the pepper plants (Park and Kim, 1992). Among them, anthracnose on the fruits was assumed to be mainly due to *C. gloeosporioides*. Recently, *C. acutatum* was identified as a major pathogen in several crop plants, although it had ben identified as a saprophytic plant pathogen having weak pathogenicity. These two species have differences in conidia morphology and growth rate. The isolates of *C. gloeosporioides* were reported to grow fast at 32°C and produce cylindrical conidia having rounded ends (Berstein et al., 1995; Freeman et al., 1996; Freeman et al., 2000; Moriwaki et al., 2002). On the contrary, the isolates of *C. acutatum* grew more slowly at 32°C and made conidia pointed on at least one end (Berstein et al., 1995; Brown et al., 1996; Adaskaveg and Hartin, 1997; Freeman et al., 1998; Freeman et al., 2000; Talhinhas et al., 2002). They also showed different responses to benomyl, one of the benzimidazole fungicides. Sensitive isolates of *C. gloeosporioides* did not grow on the media containing benomyl of 1 μg/ml, but *C. acutatum* was not inhibited at the same concentration of the fungicide (Koenraadt et al., 1992; Yarden and Katan, 1993; Berstein et al., 1995; Freeman et al., 1996; Brown et al., 1996; Albertini et al., 1999; Peres et al., 2004).

The pathogen isolates of the pepper anthracnose were collected from Korean pepper fields in 1986 to 1988 (Kim and Park, 1988; Park and Kim, 1992). Based on conidia size, morphology, and pathogenicity, the isolates obtained from the pepper fruits were identified as either *C. gloeosporioides* or *C. acutatum* (Park and Kim, 1992). Especially, more than ninety two percent of the isolates were *C. gloeosporioides*. These results were interpreted as that the major population consists of the isolates of *C. gloeosporioides* in Korean pepper fields in the late 1980th.

The field isolates collected in years 2001 and 2002 were investigated to determine whether the pathogen population changed from the late 1980th. The newly-collected isolates showed differences from *C. gloeosporioides* in several characteristics. They were less sensitive to benomyl and grew slower than *C. gloeosporioides* at 30°C, so the isolates were considered to be *C. acutatum* (unpublished

*Corresponding author.
Phone) +82-43-261-2556, FAX) +82-43-271-4414
E-mail) htkim@cbnu.ac.kr

data). It was supported by species-specific PCR. The isolates of years 2001 and 2002 could be amplified by *C. acutatum*-specific primers, but not by *C. gloeosporioides*-specific primer set (unpublished data). All of these data indicated that the pepper anthracnose resulted from *C. acutatum* increased in the fields.

Control of the pepper anthracnose has been solely dependent upon the application of fungicides because there were no resistant pepper cultivars to the disease occurred in Korea. As the disease caused by *C. acutatum* increases in the fields, it needs to know the responses of *C. acutatum* to the fungicides that currently used to control the disease. Benomyl has been widely used in the fields. However, the field isolates of *C. acutatum* were known to be less sensitive to benomyl. This less sensitivity of *C. acutatum* to benomyl should make disease control by fungicide cost-ineffective. Therefore, it is urgent to get the information of the mechanism which gives *C. acutatum* the less sensitivity to benomyl.

In this paper, the Korean isolates of *C. acutatum* were analyzed to determine whether their less sensitivity to benomyl operates by the same mechanism as in the case of *C. gloeosporioides*. First of all, it was investigated if the *C. acutatum* isolates show less sensitivity to the other bezimidazole fungicides than benomyl as *C. gloeosporioides*. Diethofencarb, belonging to the *N*-phenylcarbamate fungicides, was also used to examine the negative cross-resistance. The nucleotide sequences of the *tub2* genes encoding β-tubulin were analyzed to determine if there were mutations on the fungicides-binding amino acids.

Materials and Methods

Fungal isolates. Table 1 shows the isolates of *C. acutatum* used in this study. The isolates were prepared from the diseased pepper fruits collected in the pepper fields nationwide. The *C. gloeosporioides* isolates were obtained from strawberry experiment station (Nonsan, Korea). A pure culture originated from a single conidium was prepared as follow. The pepper fruits having lesions were incubated at 25°C for 24 h in the plastic boxes (200×250×70 mm) in which two layers of paper towels moisteurized with 50 ml sterilized water were placed on the bottom. The conidia formed on the lesions were scrapped, and suspended in sterilized water. After adjusting the concentration of the conidia suspension to 100-200 conidia/ml, one hundred microliter was spread on the PDA (potato dextrose agar) containing 300 μg/ml streptomycin. The plates were incubated at 25°C for 2-3 days. The apex of the growing hypha from one colony was detached from the agar plate, and transferred to PDA. The isolates grown from these hyphal tips were identified, based on morphological and physiological characteristics that were suggested by Adaskaveg et al. (1997). Identification of the isolates was confirmed by species-specific amplification of the ITS region of rRNA gene (Freeman et al., 2000). Total twenty three isolates of *C. acutatum* used in these experiments were stored in 6% dimethyl sulfoxide (DMSO) in the −70°C freezer as 5 mm diameter-agar blocks having the

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

Isolates	Host	Growth[a]	Benomyl[b]	Cal-1[c]	CgINT[c]
C. acutatum					
JC24	Pepper	Slow	Grow	+	−
02CCJ1	Pepper	Slow	Grow	+	−
02CCD1	Pepper	Slow	Grow	+	−
02CCM3	Pepper	Slow	Grow	+	−
KCC7	Pepper	Slow	Grow	+	−
DD33	Pepper	Slow	Grow	+	−
CB16	Pepper	Slow	Grow	+	−
FL001	Pepper	Slow	Grow	+	−
KS21	Pepper	Slow	Grow	+	−
KS77	Pepper	Slow	Grow	+	−
JE13	Pepper	Slow	Grow	+	−
JE46	Pepper	Slow	Grow	+	−
CJ04	Pepper	Slow	Grow	+	−
CJ38	Pepper	Slow	Grow	+	−
JC41	Pepper	Slow	Grow	+	−
YS05	Pepper	Slow	Grow	+	−
YS15	Pepper	Slow	Grow	+	−
BY11	Pepper	Slow	Grow	+	−
BY52	Pepper	Slow	Grow	+	−
CY10	Pepper	Slow	Grow	+	−
CY20	Pepper	Slow	Grow	+	−
MJ08	Pepper	Slow	Grow	+	−
MJ14	Pepper	Slow	Grow	+	−
C. gloeosporioides					
KACC40690	Pepper	Fast	Not grow	−	+
2001-44	Pepper	Fast	Not grow	−	+
2001-45	Pepper	Fast	Not grow	−	+
CGF253	Strawberry	Fast	Not grow	−	+
CGF69	Strawberry	Fast	Not grow	−	+
CGF50	Strawberry	Fast	Grow	−	+
CGF56	Strawberry	Fast	Grow	−	+
CGF222	Strawberry	Fast	Grow	−	+
CGF49	Strawberry	Fast	Grow	−	+
CGF210	Strawberry	Fast	Grow	−	+
CGF215	Strawberry	Fast	Grow	−	+

[a] "Slow" indicates that the mycelial growth on PDA at 25°C was below the rate of 5 mm/day. "Fast" is faster than 8 mm/day.
[b] "Growth/no growth" was determined at the PDA having 1 μg/ml of benomyl.
[c] Internal transcribed region of rDNA was amplified with either of two specific primers and a universal primer, ITS4. Cal-1: a primer specific to *C. acutatum*; CgINT for *C. gloeosporioides*. "+" indicates that a specific amplicon was produced (498bp with Cal-1 or 491bp with CgINT). "−" means that no amplicons were produced.

mycelia. New cultures were prepared on PDA by growing an agar block from the storage when used.

The fungicides and sensitivity test. Three benzimidazole fungicides, benomyl, carbendazim, and thiophanate-methyl were chosen to investigate the responses of the *C. acutatum* isolates to benzimidazoles. The mixture of carbendazim and diethofencarb was used to determine whether the isolates showing less sensitivity to bezimidazoles also had less sensitivity to *N*-phenylcarbamates. All the technical grade of benzimidazoles tested were dissolved in DMSO, and were added to the autoclaved PDA that was cooled down to about 50°C. The final concentration of DMSO in the PDA plates was below 1%. The concentrations of the fungicides tested were adjusted into the indicated concentrations. To dissolve the mixture of carbendazim and diethofencarb, sterile distilled water was used instead of DMSO, because the mixture was a commercialized fungicide (a.i. 50%, WP). The agar block carrying the mycelia of each isolate was taken with cork borer from the edge of the colony grown on PDA in dark at 25°C for 5 days. The block was transferred to the PDA containing the fungicide tested. The diameter of the growing colony was measured after incubating at 25°C in dark for 5 days. The inhibitory activity of the fungicides was estimated, based on EC_{50} and MIC (minimum inhibitory concentration) values.

DNA isolation. Each isolate was cultured for 7 days in PD broth at room temperature with shaking (at 200 rpm). Mycelia were harvested by vacuum filtration on a Buckner funnel with Whatman filter paper #5. The mycelia were freeze-dried for 48 hr, and ground with glass rod in 15 ml Falcon tube. Lysis buffer of 1.5 ml (50 mM Tris-HCl, pH 8.0, 50 mM EDTA, 0.5 M NaCl, 1% SDS) was added to the Falcon tube, which was incubated at 65°C for 30 min. Phenol extraction mixture of 1.5 ml (phenol: chloroform: isopropyl alcohol, 25:24:1 (v/v/v)) was added to the tube. After additional 30 min incubation at 65°C, the tube was centrifuged at 3000 rpm for 20 min. One milliliter of supernatant was recovered from the centrifugation. Genomic DNA was prepared by ethanol precipitation.

PCR with species-specific primers for the identification of *Colletotrichum* spp. The oligonucleotide primers CgINT (5'-GGC CTC CCG CCT CCG GGC GG-3) and Ca1-1 (5'-CAG GGG AAG CCT CTC GCG GGC CT-3') designed based on the ITS-1 region were applied for species-specific PCR (Freeman et al., 2000). CgINT (*C. gloeosporioides* specific) and Ca1-1 (*C. acutatum* specific) were each used along with the conserved primer ITS4. Each PCR reaction (30 μl) contained 50 ng of DNA, 1 μM of each primer, and 15 μl of PCR Master mix (Promega, USA). Amplification was conducted in a program as follows: 1 cycle of 4 min at 94°C, 30 cycles of 30 sec at 94°C, 30 sec at 65°C, and 1 min at 72°C, ending with 7 min at 72°C. PCR products were separated in an agarose gel. PCR amplication was repeated at least 3 times.

Amplification and sequencing of the *tub2* genes. The *tub2* gene was amplified using primers TB2L: GYTTCC-AGATYACCCACTCC and TB2R: TGAGCTCAGGA-ACRCTGACG (Peres et al., 2004). The primers were synthesized by Bioneer Inc (Taejon, Korea). Amplification was conducted in a thermal cycler (i-cycler version3.021, Biorad, Hercules, USA) in a total reaction volume of 25 μl using the PCR kit (Bioneer Inc., Taejon, Korea). The parameters used were as follows: a hold of 2 min at 95°C, 40 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C, and a final extension for 5 min at 72°C. The amplicons were column-purified with PCR Purification Kit (Atmanbio Inc., Uiwang, Korea), and sequenced by Eugentech Inc (Taejon, Korea) using Bigdye terminator chemistry (PerkinElmer, Boston, USA). The sequences were edited and align using EditSeq and MegAlign programs (DNASTAR Inc, Madison, USA).

Results

Identification of *Colletotrichum* isolates from pepper. Except for 3 isolates among all the isolates from pepper, KACC40690, 2001-44 and 2001-45, all were identified as *C. acutatum* (Table 1). The mycelial growth of *C. acutatum* isolates from pepper was slower on PDA at 25°C than that of *C. gloeosporioides.* All of the *C. acutatum* isolates from pepper grew on PDA containing benomyl (1.0 μg/ml) intermediately, compared with the sensitive and the resistant isolates of *C. gloeosporioides.* The PCR using a primer set Ca1-1 (*C. acutatum* specific primer) and ITS4 amplified the specific fragment from the *C. acutatum* isolates, and the primer pair CgINT and ITS4 amplified species-specific fragment from the *C. gloeosporioides* isolates.

Responses of *C. acutatum* to benzimidazoles. The isolates of *C. acutatum* had EC_{50} values of 0.1 to 51 μg/ml, mainly 1 to 3.4 μg/ml, to benomyl (Table 2). The EC_{50} values were mainly between 1 and 3.4 μg/ml with several exceptions. KS77 and BY52 had high EC_{50} for benomyl, 51 and 44 μg/ml, respectively, but six isolates, KCC7, DD33, CB16, FL001, KS21, and BY11 were below 1 μg/ml. MICs of the *C. acutatum* isolates for benomyl were beyond 100 μg/ml, and the differences between isolates were not observed. In the case of *C. gloeosporioides*, the response was clearly different among two groups. One had EC_{50} below 0.1 μg/

Table 2. The responses of the isolates to benzimidazoles (μg/ml)

Isolates	Benomyl		Carbendazim		Thiophanate-methyl	
	EC_{50}	MIC	EC_{50}	MIC	EC_{50}	MIC
C. acutatum						
JC24	3	>100	0.7	>100	4.9	>100
02CCJ1	3	>100	>100	>100	5.0	>100
02CCD1	2	>100	<0.1	>100	4.0	>100
02CCM3	1	>100	<0.1	>100	3.8	>100
KCC7	0.2	>100	4.9	>100	5.0	>100
DD33	0.8	>100	0.8	>100	4.3	>100
CB16	0.1	>100	3.7	>100	4.6	>100
FL001	0.1	>100	2.5	>100	6.7	>100
KS21	0.1	>100	3.0	>100	6.7	>100
KS77	51	>100	4.9	>100	7.8	>100
JE13	1.1	>100	6.2	>100	8.8	>100
JE46	1.8	>100	4.7	>100	5.9	>100
CJ04	2.2	>100	7.7	>100	7.8	>100
CJ38	1.3	>100	1.4	>100	5.1	>100
JC41	1.1	>100	2.3	>100	4.9	>100
YS05	1.3	>100	5.5	>100	5.2	>100
YS15	1.7	>100	2.8	>100	4.2	>100
BY11	0.6	>100	2.0	>100	4.7	>100
BY52	44	>100	1.4	>100	4.1	>100
CY10	1.3	>100	7.2	>100	6.3	>100
CY20	2.2	>100	3.8	>100	6.3	>100
MJ08	3.8	>100	2.9	>100	6.3	>100
MJ14	2.2	>100	1.4	>100	7.5	>100
C. gloeosporioides						
KACC40690	0.1	1	0.1	1	1.7	10
2001-44	0.1	1	0.3	1	2.1	10
2001-45	0.1	1	0.1	1	2.1	10
CGF253	<0.1	10	<0.1	1	1.5	10
CGF69	<0.1	1	0.11	0.2	1.0	10
CGF50	138	>100	>100	>100	>100	>100
CGF56	51	>100	>100	>100	>100	>100
CGF222	59	>100	>100	>100	>100	>100
CGF49	27	>100	>100	>100	>100	>100
CGF210	68	>100	>100	>100	>100	>100
CGF215	49	>100	>100	>100	>100	>100

ml, and the other had the values beyond 27 μg/ml. This separation was also shown in MIC (Table 2). The MIC values for the group having low EC_{50} were below 10 μg/ml, but the other group had the values that were higher than 100 μg/ml. These patterns were also observed in the other benzimidazoles, carbendazim and thiophanate-methyl (Table 2). Most of *C. acutatum* isolates had EC_{50} values of 0.7 to 7.7 μg/ml and 3.8 to 8.8 μg/ml for carbendazim and thiophanate-methyl, respectively. All of the isolates showed MIC that was higher than 100 μg/ml for these fungicides. In the responses to both carbendazim and thiophanate-methyl, the isolates of *C. gloeosporioides* were divided into two groups based on different responses. One group of the isolates had EC_{50} lower than 1 μg/ml for carbendazim and 2.1 μg/ml for thiophanate-methyl, but the other higher than 100 μg/ml. MICs of both fungicides for the isolates was either lower than 10 μg/ml or higher than 100 μg/ml.

The responses of the benomyl-resistant isolates to diethofencarb. The responses of nine isolates among thirty four to diethofencarb are shown in Fig. 1. Three isolates of *C. acutatum*, JC24, 02CCJ1, and 02CCM3, were not inhibited completely by both carbendazim and the mixture of carbendazim and diethofencarb. The two fungicides did not show negative correlations in their efficacies against the *C. acutatum* isolates. The MIC valuess were higher than 100 μg/ml to both carbendazim and the diethofencarb mixture. The other 20 isolates of *C. acutatum* also showed the MICs that were higher than 100 μg/ml to both fungicides (data not shown). In contrast, the isolates of *C. gloeosporioides*, CGF56, CGF222, and CGF210, responded to both fungicides exclusively. They were inhibited less than 50% by even high concentration of carbendazim, 100 μg/ml. However, they were completely inhibited by 10 μg/ml of the diethofencarb mixture. Their EC_{50} for carbendazim were higher than 100 μg/ml, but they had relatively low values for the diethofencarb mixture, 0.55, 0.48, and 0.48 μg/ml, respectively. This exclusive responses to both fungicides were observed from the other 3 isolates of *C. gloeosporioides* resistant to benzimidazoles (data not shown). The isolates of *C. gloeosporioides*, KACC40690, 02CCJ1, and 02CCM3, were inhibited 100% by carbendazim, so that they were inhibited 100% by the diethofencarb mixture. The other two isolates having low MIC of carbendazim were 100% inhibited by the mixture (data not shown).

Analyses of the *tub2* genes from the isolates resistant to benzimidazoles. A part of exon 6 of the *tub2* gene was sequenced from each isolate. It was determined whether the *C. acutatum* isolates had the base substitution(s) resulting in the non-synonymous codons. The amino acids between 136th to 284th were compared with those of *C. gloeosporioides*. There were several base substitutions were observed in the DNA sequences between *C. acutatum* and the benzimidazole-sensitive isolates of *C. gloeosporioides*, but none of them were non-synonymous codons. The amino acids at the positions of 165, 167, 198, 200, 237, 241, and 250 were alanine, phenylalanine, glutamate, phenylalanine, threonine, argine, leucine, respectively. No amino acid substitutions were observed between *C. acutatum* and the benomyl-sensitive *C. gloeosporioides*. On the contrary, the benzimidazol-resistant isolates of *C. gloeosporioides* had alanine at the position of 198 instead of glutamic acid. All the other amino acids were synonymous to the sensitive

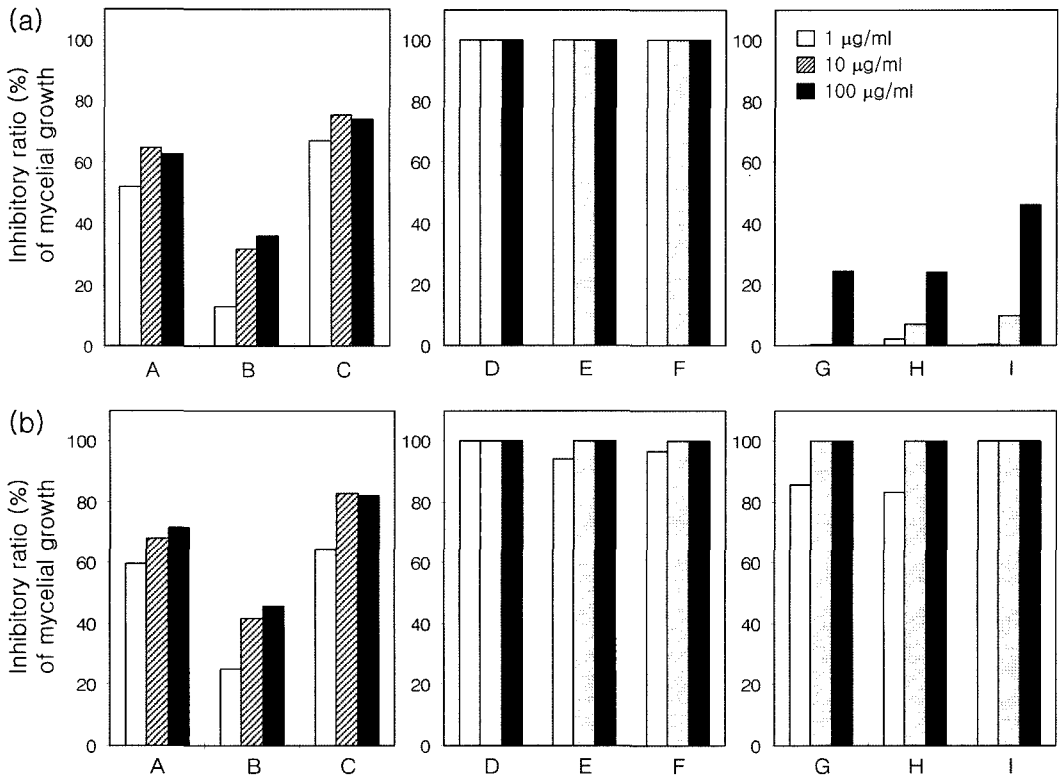

Fig. 1. Responses of the isolates to carbendazim (a) and the mixture of carbendazim and diethofencarb (b). Y axis indicates the inhibition ratio (%) of mycelial growth: (1-(colony diameter at treatment/colony diameter at control))×100 A: JC24, B: 02CCJ1, C: 02CCM3, D: KACC40690, E: CGF253, F: CGF69, G: CGF56, H: CGF222, and I: CGF210.

isolates of *C. gloeosporioides*.

Discussion

The responses of the isolates to fungicides were estimated by measuring EC_{50} and MIC values. However, those values were frequently contradictory to each other. EC_{50} of benomyl for CB16 was as low as 0.1 μg/ml, but its MIC was higher than 100 μg/ml. Thus, the range and the pattern of both values, EC_{50} and MIC, and their correlation were cautiously analyzed to determine resistance/sensitivity of the isolates to benzimidazoles. In these experiments, the isolates of *C. acutatum* showing a high MIC to benomyl generally showed the tendency that they also had high MIC to both carbendazim and thiophanate-methyl. These results confirmed the previous reports that the isolates of *C. acutatum* were less sensitive to benzimidazoles. This response of *C. acutatum* to benzimidazoles has difference from the resistance of *C. gloeosporioides* to benomyl. The former is inherently less sensitive, which is not classified to be either sensitive (wild type) or resistant (mutant) as shown in the latter. The other difference is that the less sensitivity of *C. acutatum* is a quantitative trait, whereas the resistance of *C. gloeosporioides* is qualitative one. In other words, the EC_{50} values of benzimidazoles for *C. acutatum* showed continuous distribution from 0.1 μg/ml to 55 μg/ml, whereas *C. gloeosporioides* has only two different EC_{50}: one below 1 μg/ml and the other beyond 27 μg/ml. There was some exception like KS77 and BY52 in their response to benomyl. Several explanations may be possible for the differences between these pathogens. For example, the less sensitivity of *C. acutatum* may result from not the specific mutation on the fungicide target site, but the other mechanism. This experiment did not exclude the possibility that the resistance of two *Colletotrichum* species is based on the same mechanism. The response of *C. acutatum* to the mixture of carbendazim and diethofencarb showed variation from those of *C. gloeosporioides* (Fig. 1). Highly benzimidazole-resistant isolates of *C. gloeosporioides* had negative cross-resistance to diethofencarb. However this negative correlation was not observed in *C. acutatum*. The less sensitive isolates of *C. acutatum* to carbendazim showed an intermediate response to the diethofencarb mixture, neither highly resistant nor highly sensitive. This can be explained by the possibility that the negative correlation in *C. acutatum* does not exist in the response to the carbendazim and diethofencarb mixture. This suggests that the resistance of *C. acutatum* to benzimidazoles is dependent

upon different mechanism from that of *C. gloeosporioides*.

The benzimidazole-resistance was reported in other organisms to be correlated with an amino acid substitution (s) on β-tubulin, the target site of the fungicide (Albertini et al., 1999; Orbach et al., 1986). At least one of seven amino acids was replaced in the mutant isolates showing the resistance to benzimidazole. For example, the resistant isolates of *Venturia inaequalis* substitute alanine for glutamate at position 198 of *TUB2* (Koenraadt et al., 1992). The resistant isolates of *Botrytis cinerea* have substitutions at the amino acids 198 and 200 (Yarden and Katan, 1993). In addition, the amino acid changes at 165, 167, 237, 241, 250, and 350 were observed in resistant isolates that were mutated in laboratory (Davidse and Ishii, 1995). With the 15 selected isolates of *C. acutatum* and *C. gloeosporioides*, the amino acid substitutions were not analyzed on these positions in details (Peres et al., 2004). Sequence analyses also reflect the difference in the resistance mechanism (Table 3). All the highly resistant isolates of *C. gloeosporioides* had alanine at 198 position of β-tubulin. However, all the less sensitive isolates of *C. acutatum* still had glutamic acid at the same position as the sensitive isolates of *C. gloeosporioides*. This means that the less sensitivity of *C. acutatum* to benzimidazoles is not due to the amino acid substitution from glutamate to alanine at the position 198 of β-tubulin. This conclusion is supported by the recent report that even the mutant isolates of *C. acutatum* having lysine at position 198 did not result in highly resistance to benzimidazols. Other mechanisms may involve in the less sensitivity of *C. acutatum* to benzimidazoles.

The responses of *C. acutatum* are not assumed to be specific to both benzimidazols and *N*-phenylcarbamates as in the case of *C. gloeosporioides*. This reflects that the fungicides partially inhibit the growth of *C. acutatum*. Under this assumption, a plausible mechanism could be suggested that the sufficient amount of fungicides can not bind to the target sites of *C. acutatum*. Many mechanisms can be considered to explain the less sensitivity of *C. acutatum*. One of the effective approaches to understand it could be through mutation analysis using the mutant isolates that are sensitive to the fungicides.

Acknowledgment

This work was supported by a grant (Code 20050301-034-408-007-05-00) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

References

Adaskaveg, J. E. and Hartin, R. J. 1997. Characterization of *Colletotrichum acutatum* isolates causing anthracnose of almond and peach in California. *Phytopathology* 87:979-987.

Albertini, C., Gredt, M. and Leroux, P. 1999. Mutations of the β-tubulin gene associated with different phenotypes of benzimidazole resistance in the cereal eyespot fungi *Tapesia yallundae* and *Tapesia acuformis*. *Pest. Biochem. Physiol.* 64:17-31.

Bernstein, B., Zehr, E. I. and Dean, R. A. 1995. Characteristics of *Colletotrichum* from peach, apple, pecan, and other hosts. *Plant Dis.* 79:478-482.

Brown, A. E., Sreenivasaprasad, S. and Timmer, L. W. 1996. Molecular characterization of slow-growing orange and key lime anthracnose strains of *Colletotrichum* from citrus as *C. acutatum*. *Phytopathology* 86:523-527.

Freeman, S., Minz, D., Jurkevitch, E., Maymon, M. and Shabi, E. 2000. Molecular analyses of *Colletotrichum* species from almond and other fruits. *Phytopathology* 90:608-614.

Davidse, L. C. and Ishii, H. 1995. Modern selective fungicides ed. by H. Lyr, Gustav Fisher Verlag, Jena, Stuttgart, New York, pp. 305-322.

Freeman, S., Katan, T. and Shabi, E. 1996. Characterization of *Colletotrichum gloeosporioides* isolates from avocado and almond fruits with molecular and pathogenicity tests. *Appl. Environ. Microbiol.* 62:1014-1020.

Freeman, S., Katan, T. and Shabi, E. 1998. Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. *Plant Dis.* 82:596-605.

Kim, C. H. and Park, K. S. 1998. A predictive model of disease progression of red-pepper anthracnose. *Korean J. Plant Pathol.* 4:325-331.

Koenraadt, H., Somerville, S. C. and Jones, A. L. 1992. Characterization of mutations in the bete-tubulin gene of benomyl-resistant field strains of *Venturia inaequalis* and other plant pathogenic fungi. *Phytopathology* 82:1348-1354.

Moriwaki, J., Tsukiboshi, T. and Sato, T. 2002. Grouping of *Colletotrichum* species in Japan based on rDNA sequences. *J. Gen. Plant Pathol.* 68:307-320.

Orbach, M. J., Porro, E. B. and Yanofsky, C. 1986. Cloning and characterization of the gene for bete-tubulin from a benomyl-resistant mutant of *Neurospora crassa* and its use as a dominant selectable marker. *Mol. Cell Biol.* 6:2452-2461.

Park, K. S. and Kim, C. H. 1992. Identification, distribution and etiological characteristics of anthracnose fungi of red pepper in Korea. *Korean J. Plant Pathol.* 8:61-69.

Peres, N. A. R., Souza, N. L., Peever, T. L. and Timmer, L. W. 2004. Benomyl sensitivity of isolates of *Colletotrichum acutatum* and *C. gloeosporioides* from citrus. *Plant Dis.* 88:125-130.

Talhinhas, P., Sreenivasaprasad, S., Neves-Martins, J. and Oliveira, H. 2002. Genetic and morphological characterization of *Colletotrichum acutatum* causing anthracnose of lupins. *Phytopathology* 92:986-996.

Yarden, O. and Katan, T. 1993. Mutation leading to substitutions at amino acids 198 and 200 of beta-tubulin that correlate with benomyl-resistance phenotypes of field strains of *Botrytis cinerea*. *Phytopathology* 83:1478-1483.