Plant Pathol. J. 22(2) : 147-151 (2006)

The Plant Pathology Journal

Incidence and Distribution of Virus Diseases on Cucumber in Jeonnam Province During 1999-2002

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(Received on April 17, 2006; Accepted on May 15, 2006)

Disease incidences of cucumber virus diseases in Jeonnam province were 52.5%, 16.1%, 35.2%, and 50.9% in 1999, 2000, 2001, and 2002, respectively. Rod- and flexuous rod-shaped virus particles were observed with the frequencies of 63.2% and 10.5%, respectively from the samples collected in 1999 under EM observation. Rod-shaped virus particles are considered as tobamovirus while flexuous rod shaped particles are considered as potyviruses. To further confirm their nature, total of 312 diseased virus samples were collected from 2000 to 2002, and tested by RT-PCR. Disease incidences of tobamoviruses including *Cucumber green mottle mosaic virus* and *Kyuri green mottle mosaic virus* were 48.7% and 3.8%, respectively while those of potyviruses including *Zucchini yellow mosaic virus*, *Papaya ringspot virus*, and *Watermelon mosaic virus* were 15.7%, 9.3%, and 5.1%, respectively. Interestingly, *Cucumber mosaic virus* was hardly detected. About 5.8% of tested samples were infected with more than one virus. Tobamovirus infection was consistently observed from September to December regardless of planting time, whereas infection of potyviruses was observed in many cucumber cultivating areas where it was planted in September and October.

Keywords : cucumber, disease incidence, potyviruses, RT-PCR, tobamoviruses

Cucumber (*Cucumis sativus* L.) is an important vegetable crop in Jeonnam province. It occupied approximately 787 ha with nearly 90% of cultivated area in plastic-houses in Jeonnam province. An estimated production of cucumber in 2004 was 83,886 tons (Ministry of Agriculture and Forestry, 2005). At least 9 different viruses were reported to infect cucumber worldwide (Antignus et al., 2001; Dias and Mckeen, 1972; Francki et al., 1979, 1986; Hollings et al., 1975; Lisa and Lecoq, 1984; Purcifull et al., 1984a, b; Weber, 1986). Among them, five different viruses including *Cucumber mosaic virus* (CMV, cucumovirus), *Cucumber green mottle mosaic virus* (CGMMV, tobamovirus), *Kyuri green mottle mosaic virus* (KGMMV, tobamovirus), *Watermelon mosaic virus* 2 (WMV2, potyvirus), and *Zucchini yellow mosaic virus* (ZYMV, potyvirus) have been reported to infect cucumber in Korea (Cheon et al., 2000; Ko et al., 2000, 2004a, b; Lee and Lee, 1981; Lee et al., 2001). An outbreak of CGMMV infection was occurred in Korea, infecting cucumber cultivation area of 5.3 ha in 2001 (Choi, 2001). However, there is no available information on the virus disease incidences, periodical disease severity, and the potential economic impact of virus infection on cucumber production and thus we do not know exactly what is the most important viruses infecting cucumber in Korea. To determine the most prevalent viruses infecting commercially grown cucumber in Jeonnam province, we collected samples from five different major cucumber cultivating areas from 1999 to 2002 and confirmed virus disease incidences using symptomatology, EM and RT-PCR analyses.

Materials and Methods

Survey of cucumber viruses. Surveys were conducted from January to April during four years (from 1999 to 2002) in major cucumber cultivating areas in Jeonnam province including Gangjin, Goheung, Gwangyang, Gurye, Boseong, Suncheon, and Yeosu (Fig. 1). Disease incidence was evaluated based on plants showing virus-like symptoms by observing at least 300 plants per plastic house. Samples showing virus-like symptoms were collected and further examined for virus particles using electron microscopy (EM). Leaf-dip (Horne et al., 1963) was carried out

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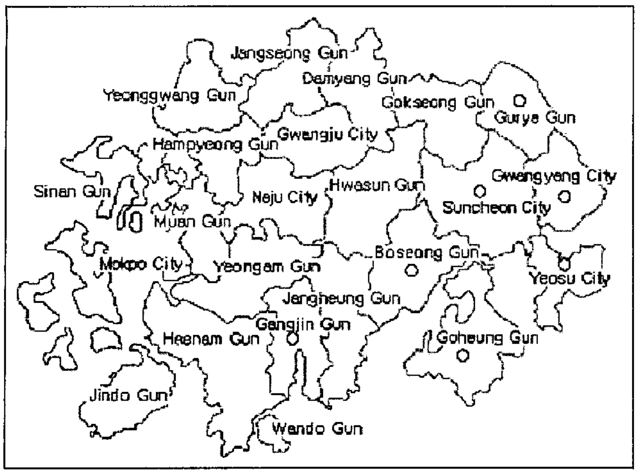

Fig. 1. Surveyed districts of virus diseases on cucumber in Jeonnam province.

with 2% phosphotungstic acid (PTA, pH 7.2) and was examined for virus in an EM (JEM-1010; JEOL Ltd., Japan).

Total RNA extraction and RT-PCR. Total RNAs were extracted from 20 mg of freeze-dried samples using RNAgents® Total RNA Isolation System kit (Promega Co., USA) according to the manufacture's protocols. Primers were designed based upon sequence information given in the literature (Jin et al., 2003; Lee et al., 2003). The size and relative quality of the RNAs were determined by electrophoresis in an 1.2% agarose gel using molecular mass size marker. Reverse transcription (RT) was carried out in a final 20 μL volume obtained by adding 1 μL total RNA (2.5 ng/μL), 25 pmole (1 μL) of the downstream primer, 1×RT buffer (50 mM Tris-HCl, 50 mM KCl, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM spermidine), 2 mM dNTP, 20 U RNase inhibitor, and 9 U AMV reverse transcriptase (Promega Co., USA) and made up to volume with DEPC-dH_2O. RT was carried out at 42°C for 30 min, and was denatured by heating at 95°C for 5 min. When RT was completed, total 80 μL of 25 pmole (1 μL) of the upstream primer, 2.5 U Taq DNA polymerase (Promega Co., USA), 1× PCR buffer (10 mM Tris-HCl, 50 mM KCl), 2 mM $MgCl_2$, 0.4 mM dNTP, were added. Mixtures were then amplified in a T-

Table 1. Occurrence of virus diseases on cucumber in Jeonnam province[a]

Year	District	No. of fields		Diseased fields (%)	Disease incidence (%)[b] (Range)
		investigated	diseased		
1998/1999	Boseong	28	22	78.6	51.7 (0.5~100)
	Suncheon	43	27	62.8	64.0 (0~100)
	Gurye	14	3	21.4	43.1 (5~90)
	Gwangyang	6	4	66.7	51.1 (16.7~77.5)
	Subtotal	91	56	61.5	52.5 (0.5~100)
1999/2000	Boseong	10	2	20.0	0.4 (0.1~0.7)
	Suncheon	11	2	18.2	15.2 (9.6~20.8)
	Gurye	13	5	38.5	37.3 (0.8~90.0)
	Gwangyang	5	2	40.0	13.8 (2.7~24.9)
	Gangjin	5	2	40.0	13.8 (90.0~100)
	Subtotal	44	13	29.5	16.1 (0.1~100)
2000/2001	Boseong	10	0	0	–
	Suncheon	28	18	64.3	10.0(0.2~65.3)
	Gurye	25	11	44.0	46.6 (5.1~96.7)
	Gwangyang	14	6	42.9	46.9 (12.5~59.4)
	Gangjin	5	0	0	–
	Goheung	7	5	71.4	39.0 (8.4~100)
	Yeosu	9	3	33.3	33.3 (19.8~46.9)
	Subtotal	98	43	43.9	35.2 (0.2~100)
2001/2002	Boseong	13	2	15.4	54.9 (47.3~62.5)
	Suncheon	30	3	10.0	43.3 (13.5~72.5)
	Gurye	13	2	15.4	33.4 (21.5~45.3)
	Gwangyang	15	1	6.7	71.9
	Gangjin	6	0	0	–
	Subtotal	77	8	10.4	50.9 (13.5~72.5)

[a] Cucumber was planted from September to October and was investigated from February to April each year.
[b] Disease incidence represents the percentage of mean number of infected plants per total plants in a field. More than 300 plants were investigated in each field.

gradient Thermocycler (Biometra Co., Germany) for a total 40 cycles. Each cycle included a denaturing step at 94°C for 60 sec, an annealing step at 60°C for 60 sec, and extension step at 72°C for 80 sec, and finally kept at 72°C for 7 min. PCR products were analyzed by an 1.2% agarose gel electrophoresis, stained with ethidium bromide, and visualized using a UV transilluminator.

Results

Survey of cucumber viruses. Virus disease incidence in cucumber cultivating fields were 52.5% in Jeonnam in 1999 with higher disease incidence in Boseong and Suncheon areas. In 2000, it was decreased to 16.1% in 2000, and was similar in most surveyed areas except Boseong and Gurye with 0.4% and 37.3%, respectively. It was increased to 35.2% in 2001 with severe incidence in Goheung, Gwangyang, Gurye, and Suncheon areas and was increased to 50.9% in 2002 (Table 1). Similar rate of incidences were also observed when we looked at the rate of diseased fields. Disease field incidences of cucumber virus infection in Jeonnam province were 61.5%, 29.5%, and 43.9% in 1999, 2000, and 2001, respectively. In 2002, however, diseased field incidence was decreased to 10.4% whereas disease incidence was 50.9% (Table 1).

Detection of viruses. Eighty one out of the 95 samples contained virus particles when examined under EM. Rod and flexuous rod shaped virus particles were observed from 60 and 10 samples, respectively. Interestingly, 11 samples contained both rod and flexuous rod shaped virus particles (Table 2). To further characterize the nature of rod and flexuous rod shaped viruses, RT-PCR was employed using specific oligonulceotide primer sets for CGMMV, KGMMV, ZYMV, *Papaya ringspot virus* (PRSV), WMV, and CMV. A total of 312 samples collected during 2000-2002 were used. A CGMMV- and KGMMV-specific DNA fragments were amplified from 152 and 12 samples, respectively. Potivirus-specific DNA fragments for ZYMV, PRSV, and WMV were amplified from 48, 30, and 16 samples, respectively. CMV-specific DNA fragment was detected from only 3 samples. Double virus infection was detected in all of the samples by RT-PCR. The nature and specificity of amplified DNA fragments for each virus was confirmed by DNA sequencing analysis (data not shown). No virus-specific DNA fragment was amplified from 33 samples. It is possible that they might be infected with the other virus(es) which we did not test. In general, CGMMV was the most prevalent virus infecting cucumber in Jeonnam province, while ZYMV was the most prevalent virus infecting cucumber in 2002. Altogether, these results indicated that the seed-transmissible tobamoviruses including CGMMV and KGMMV were prevalent in 2000 and 2001 and decreased in 2002, whereas the disease incidence caused by the aphid transmissible potyviruses including ZYMV, PRSV, and WMV were low in 2001 and 2002 and significantly increased in 2002 (Table 3).

Table 2. EM observation of cucumber samples showing virus-like symptoms collected from plastic houses

Year	No. of samples	Detection rate (%)	R[a]	R+F	F[b]
1999	95	85.3	60	11	10

[a] Rod-shaped virus particle.
[b] Flexuous rod-shaped virus particle.

Occurrence of virus diseases with the planting time. To determine virus disease incidence with the cucumber planting time, cucumber plants were transplanted from September to December. Field samples were collected from February to April. PRSV was found in five samples in two fields when cucumber plant was transplanted at September. No other virus was detected (Table 4). However, CGMMV, WMV, ZYMV, and PRSV were found in 24, 7, 8, and 1 of forty-four samples collected from thirteen fields where cucumber plants were transplanted at October. Three samples were infected with two different viruses. When cucumber plants were transplanted at November or December, only tobamovirus infections by CGMMV and KGMMV were observed. CGMMV and KGMMV were detected in 25 and 1 out of 26 samples, respectively, collected from seven

Table 3. RT-PCR detection of virus diseases occurred on cucumber in Jeonnam province from 2000 to 2002

Year	No. of samples tested	No. of samples detected with[a]													
		Nd	C	CG	KG	W	Z	P	C+P	CG+P	CG+Z	P+W	Z+W	Z+KG	CG+Z+P
00	55	0	0	34	8	2	2	5	0	2	0	0	1	0	1
01	208	18	1	108	4	14	31	19	2	2	5	1	2	1	0
02	49	15	2	10	0	0	15	6	0	0	0	0	1	0	0
Total	312	33	3	152	12	16	48	30	2	4	5	1	4	1	1

[a] C, CMV; CG, CGMMV; KG, KGMMV; W, WMV; Z, ZYMV; P, PRSV; Nd, not detected.

Table 4. RT-PCR detection of virus diseases occurred on cucumber based upon planting times

Planting time[a]	No. of surveyed fields	No. of diseased fields	No. of samples detected with[b]									
			total	C	CG	KG	W	Z	P	CG+Z	KG+Z	Z+W
Sep.	5	2	5	0	0	0	0	0	5	0	0	0
Oct.	31	13	44	0	24	0	7	8	1	1	1	1
Nov.	12	7	26	0	25	1	0	0	0	0	0	0
Dec.	5	2	6	0	6	0	0	0	0	0	0	0

[a] Cucumber plant samples were collected from February to April in 2001.
[b] C, CMV; CG, CGMMV; KG, KGMMV; W, WMV; Z, ZYMV; P, PRSV.

fields transplanted at November. Only CGMMV was detected in 6 samples collected from six fields when transplanted at December. In general, infections of tobamoviruses (CGMMV and KGMMV) were prevalent when cucumber plants were transplanted after October while thoses of potyviruses (WMV, ZYMV, and PRSV) were observed in cucumber fields transplanted only at September and October (Table 4).

Discussion

This survey revealed that CGMMV, ZYMV, and PRSV are the most common viruses causing severe symptoms and yield loss on cucumber. It indicated that incidence of diseased field was different according to the surveyed year, because CGMMV was dramatically decreased since 2002. The rate of diseased field was commonly high in 1999 except Gurye area. The rate of the disease field incidence was changed dramatically depending on the CGMMV-infection in fields. CGMMV-infection was greatly decreased in 2002 and thus low diseased field incidence was observed (Table 1). Disease incidence of the seed-borne CGMMV-infection slowly decreased since the severe occurrence in 1999. EM examination of samples in 1999 revealed that rod-shaped particles were tobamoviruses including CGMMV or KGMMV that are seed-transmissible (Francki et al., 1986; Hollings et al., 1975). Significant decrease of tobamovirus disease incidence might be due to the fact that the strict sterilization of cucumber seeds by seed companies since then.

Flexuous rod-shaped particles examined under EM were potyvirus particles including WMV, ZYMV, and PRSV as they were confirmed by RT-PCR. This is in agreement with the previous reports (Lisa and Lecoq, 1984; Purcifull et al., 1984a, b). Interestingly, the disease incidence caused by the CMV was very low. No CMV related virus particle was observed under EM and only 3 samples (out of 312; 0.96%) were infected with CMV. Ullman et al. (1991) reported that ZYMV-infection was predominated in Hawaiian. They also mentioned that CMV and WMV were no longer present and that PRSV rarely infect cucumber. In contrast, PRSV and ZYMV were the most prevalent viruses infecting cucumber in Brazil, whereas *Zucchini lethal chlorosis virus*, CMV, and WMV were lowly found (Yuki et al., 2000). Similar infection pattern of potyviruses has also been observed in Jeonnam province. ZYMV is the most prevalent virus, followed by PRSV and WMV, but CMV was rarely found.

The occurrence of a tobamovirus-infections caused by CGMMV and KGMMV was not significantly influenced by the planting times, whereas the occurrence of a aphid-borne potyviruses, WMV, ZYMV, and PRSV, was observed only in cucumber plants planted in September and October. These results suggest that the disease incidence caused by the aphid-transmissible potyviruses might closely relate with the population density of virus-transmissible aphid vectors. Therefore, these regions will require an aggressive program of integrated tactics that repel aphids, such as stylet oils and reflective mulches when transplanted earlier (Lobenstein et al., 1966; Mokkouk et al., 1986; Skotrand et al., 1963; Summers et al., 1995; Vega et al., 1992; Yudin et al., 1990). Since high infection rates occurred only in the early planting, the late planting may be a successful strategy for minimizing the impact of epidemics of aphid-borne viruses in these regions.

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