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Note

The Incidence of Virus Diseases on Melon in Jeonnam Province during 2000-2002

Sug-Ju Ko[1]*, Yong-Hwan Lee[2], Myoung-Soo Cho[1], Jin-Woo Park[2], Hong-Soo Choi[2], Geun-Cheol Lim[1] and Kook-Hyung Kim[3]

[1]*Vegetable Experiment Station, Jeonnam Agricultural Research and Extension Services, Kurye 542-821, Korea*
[2]*Depart of Plant Pathology, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea*
[3]*Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea*

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The occurrence and relative incidence of viruses including *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Papaya ringspot virus* (PRSV), and *Watermelon mosaic virus* (WMV), *Cucumber green mottle mosaic virus* (CGMMV), *Kyuri green mottle mosaic virus* (KGMMV), and *Melon necrotic spot virus* (MNSV) were surveyed from main melon (*Cucumis melo* L.) production areas in Jeonnam province during 2000-2002. Virus disease incidences of melon cultivating fields were 0% and 11% in spring and fall 2000; 40%, 2.1%, and 8.8% in spring, summer, and fall 2001; and 6.3% in spring 2002 in main cultivated areas in Jeonnam province, respectively. Field disease incidences of melon virus infections were 0% and 18.8% in spring and fall 2000; 50%, 38.5%, and 82.6% in spring, summer, and fall 2001; and 47.4% in spring 2002, respectively. Total of 101 melon samples showing typical disease symptoms were collected from 2000 to 2002 and tested for virus infection by RT-PCR. Potyvirus-specific DNA fragments for WMV, ZYMV, and PRSV were amplified from 46, 5, and 4 samples, respectively. MNSV specific DNA fragment was amplified from 18 samples. CMV-specific DNA fragment was detected from only 3 samples.

Keywords **:** disease incidence, melon, MNSV, potyviruses, RT-PCR

Cucurbit crops are of great economic importance in Jeonnam province. Melon (*Cucumis melo* L.) was cultivated approximately 478 ha in 2005 in Korea and Jeonnam province covers a significant portion (38.7%) and thus plays an important role in overall melon production in Korea (Ministry of Agriculture and Forestry, 2006). At least 13 different viruses were reported to infect melon worldwide (Abou-Jawdah et al., 2000; Alonso-Prados et al., 2003; Avgelis, 1985; Boubourakas et al., 2006; Daryono et al., 2005; Gonzalez-Garza et al., 1979; Graftin-Cardwell et al., 1996; Kato et al., 2000; Kubo et al., 2005; Luis-Arteaga et al., 1998; Mahgoub et al., 1997; Raychaudhuri et al., 1978; Yuki et al., 2000). Among them, 3 different viruses including CGMMV (tobamovirus), *Melon necrotic spot virus* (MNSV, carmovirus), and *Papaya ring spot virus* (PRSV, potyvirus) have been reported to infect Melon in Korea (Cho et al., 2005; Choi et al., 2001; Choi, 2003).

Occurrence of virus and virus-like diseases can limit production. Investigations of incidence and distribution of melon viruses are very important in developing diagnostic systems and appropriate control measures. However, there is no available information on the virus disease incidences, periodical disease severity, and the potential economic impact of virus infection on melon production and thus we do not know exactly what is the most important viruses infecting melon in Korea. To determine the most prevalent viruses infecting commercially grown melon in Jeonnam province, we collected samples from major melon cultivating areas from 2000 to 2002 and confirmed virus disease incidences using RT-PCR analyses.

Survey of virus diseases. Surveys were conducted during three years (2000-2002) in major melon cultivating areas in Jeonnam province including Naju city, Damyang, Gokseong, and Hwasun district (Fig. 1). Virus disease incidences were surveyed both on spring (April to June) and on fall (September to October) each year. 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 tested by reverse transcription-polymerase chain reaction (RT-PCR).

Virus disease incidence of melon-cultivating fields were 0% and 11% in main cultivated area in Jeonnam in spring and fall 2000, respectively. It was 40%, 2.1%, and 8.8% in spring, summer, and fall 2001 and 6.3% in spring 2002 in main cultivated areas in Jeonnam province, respectively. Field disease incidences of melon virus infections were 0%

*Corresponding author.
Phone) +82-61-781-5230, FAX) +82-61-781-5231
E-mail) kosj@jares.go.kr

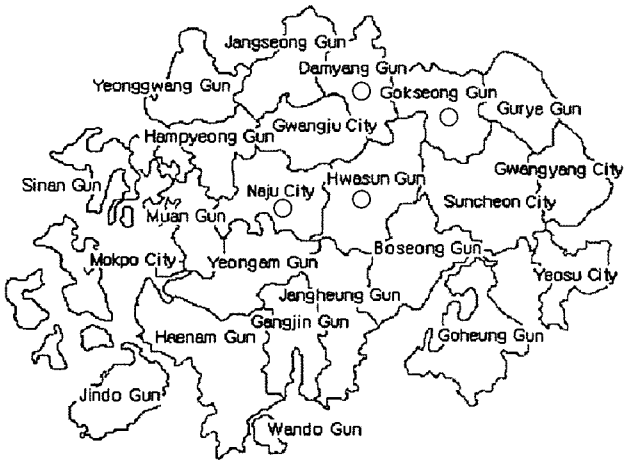

Fig. 1. Surveyed districts of virus diseases on melon in Jeonnam province.

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

Year	Investing times	No. of fields		Diseased fields (%)	Disease incidence (%)[b] (Range)
		investigated	diseased		
2000	Spring	10	0	0	0
	fall	32	6	18.8	11.0(0.5-49.8)
2001	Spring	2	1	50.0	40.0
	Summer	13	5	38.5	2.1(0.6-3.3)
	fall	23	19	82.6	8.8(0.4-45.5)
2002	Spring	19	9	47.4	6.3(0.1-26.5)

[a]Spring was investigated from April to June, summer from June to September, fall from September to November, winter in January each year.

[b]Disease incidence represent the percentage of mean number of infected plants per total plants in a field. More than 300 plants were investigated in each field.

and 18.8% in spring and fall 2000; 50%, 38.5%, and 82.6% in spring, summer, and fall 2001; and 47.4% in spring 2002, respectively (Table 1). Typical symptoms associated with virus infections include mosaic, chlorotic mottle, vein clearing, vein banding, severe mosaic with CMV, PRSV, WMV, and ZYMV infections while necrotic spot and stem necrosis were observed with MNSV infection.

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. The quality and relative concentrations of transcripts were checked by electrophoresis on 1.2% (w/v) agarose gel at 4°C and visualized by ethidium bromide staining. Specific oligonucleotide primers for PRSV, WMV, ZYMV, KGMMV, CGMMV, CMV, and MNSV were designed based upon sequence information given in the literature (Table 2). A RT-PCR was carried out with one cycle at 42°C for 30 minutes, 95°C for 5 minutes and 35 cycles of PCR amplification using the step program (94°C, 60 seconds; 60°C, 60 seconds; 72°C, 80 seconds) followed by a final extension at 72°C for 7 minutes. Five μl of the amplified DNA fragments were separated by electrophoresis on a 1.2% agarose gel in 1× Tris-acetate-EDTA (TAE) buffer and stained with ethidium bromide. A total of 101 samples that were collected from melon during 2000-2002 were assayed. As might be suspected, primer sets for WMV,

Table 2. The sense and antisense primer pairs used in this study

Target	Primer pairs[a]	Sequence	Length	Product(bp)	References
CMV	CMR3-C30(−)	CCACACGGTAGAATCAAAT	19	850	Lee et al. (2003)
	CMR3-N40(+)	GCTCGCCTGTTGAAGTCGCA	20		
CGMMV	CGMM-C60(−)	AATTAAGTAAAGTCCTGACG	20	609	
	CGMM-N30(+)	ATGGAACGTACCGGAATC	18		
KGMMV	KGMM-C10(−)	GAGAACTTACAGATAG	16	376	
	KGMM-N60(+)	AGTCGCGCATTGCTGCTTTGAT	22		
WMV	WM2-C20(−)	CTTATAACGACCCGAAATGCTA	22	321	
	WM2-N50(+)	AGTCCGTATATGCCTAGAT	19		
ZYMV	ZYM-C10(−)	AGGCTTGCAAACGGAGTCTAAT	22	500	
	ZYM-N50(+)	TATATAGAGATGAGAAATGCAGA	23		
PRSV	PRS-C20(−)	TCACTGTAAAATAGAAGCGGT	21	600	Jin et al. (2003)
	PRS-N60(+)	CAATTTGAGAAGTGGTATGAG	21		
MNSV	MNS-C10(−)	CTCCATAAGCGCCAAGCAACC	21	485	Kim et al. (2005)
	MNS-N40(+)	AGCGGGGGAAAACAGAAGAA	20		

The primers were selected after the alignment of the nucleotide sequences from the known isolates of each virus. [a](−), downstream primer; (+), upstream primer.

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

Year	Investing times	No. of samples tested	No. of samples detected with[a]												
			Nd	C	CG	W	Z	P	M	M+P	M+W	W+P	Z+P	Z+W	W+Z+P
2000	Fall	14	0	0	0	4	0	4	5	1	0	0	0	0	0
2001	Spring	3	0	0	0	0	0	0	3	0	0	0	0	0	0
	Summer	12	0	0	0	8	0	0	0	0	0	4	0	0	0
	Fall	62	0	3	0	33	5	0	2	0	2	13	2	1	1
2002	Spring	10	1	0	0	1	0	0	8	0	0	0	0	0	0

[a]C, *Cucumber mosaic virus*; CG, *Cucumber green mottle mosaic virus*; M, *Melon necrotic spot virus;* W, *Watermelon mosaic virus*; Z, *Zucchini yellow mosaic virus*; P, *Papaya ringspot virus;* Nd, not detected.

ZYMV, and PRSV amplified expected size DNA fragments from 46, 5, and 4 samples, respectively. In addition, primer sets for MNSV and CMV also amplified specific DNA fragment from 18 and 3 samples, respec- tively. The other primer sets for CGMMV and KGMMV did not amplify any specific DNA fragment indicating no infection of CGMMV and KGMMV. Double virus infec- tion was detected in 23 samples of all samples that tested positive by RT-PCR, and triple virus infection was detected in only one sample. Double infection with WMV + PRSV accounted for 73.9%. In some case, non-specific bands are also detected but the size and the amount of amplified DNAs were significantly different. The nature and specificity of amplified DNA fragments for each virus was confirmed by DNA sequencing analysis (data not shown). In general, WMV and MNSV were the most prevalent virus infecting melon in Jeonnam province. Altogether, these results indicated that the seed-borne and soil-borne MNSV were prevalently infecting melon plants both in spring and fall every year. In contrast, the disease incidence caused by the aphid transmissible potyviruses including ZYMV, PRSV, and WMV were high only in summer and fall 2000 and fall 2001 (Table 3).

This survey revealed that WMV and MNSV are the most common viruses causing severe symptoms and yield loss on melon. Recent surveys of melon fields from the Central Valley of California indicate that WMV is most prevalent virus, followed by CMV and ZYMV (Grafton-Cardwell et al., 1996). It was CMV and WMV in Spain (Alonso-Prados et al., 1997, 2003; Luis-Arteaga et al., 1998). But it was PRSV and ZYMV in Brazil (Yuki et al., 2000). Melons were more severely affected in 2001, and WMV was the most prevalent virus and widespread virus. Similar trend was apparent in cucumber infected with potyvirus (Ko et al., 2006). Virus infections caused by non-persistently transmitted viruses such as CMV, WMV, ZYMV, and PRSV were in spring, summer, and fall, but, seed- and soil-borne virus such as MNSV occurred in spring, fall, and winter. In Japan, the virus was prevalent in winter (Hibi and Fruki, 1986). It remains to be elucidated to see the effects of development condition such as weather temperature, fungal vector, and seed transmission on virus disease incidences. Based on these results, different management strategies should be utilized to reduce virus epidemics on melon depending on the growing region. If melon cultivating regions show high CMV, WMV, ZYMV, and PRSV infections, it 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; Summers et al., 1995; Yudin et al., 1990). However, seed and soil-borne virus such as MNSV infection is wide-spread, it might need introduce soil disinfection and sterilized seed. Altogether, the data reported here provide essential basic information to establish the basis for the control of virus epidemics in plastic house-grown melon in Jeonnam province.

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